Course Notes for Summer Introductory Biology (BIO 1107-1108), as taught in the summer of 2010 at Cornell University, Ithaca, New York, USA.

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Preface

A few comments on the instructional approach taken in these classes.

Every instructor has to cope with trade offs between many factors in their instruction. One constraint that I had in teaching the summer Introductory Biology (BIO 1107-1108) classes at Cornell related to the purpose of these courses. Being taught over the summer, these courses were intended to be a means for students who, for what ever reason, did not complete the regular semester introductory biology sequence at Cornell (i.e. BIO 1101-1104) to receive equivalent instruction. Thus between them BIO 1107-1108, taught over just an eight week period, had to cover the core material from the regular courses that spanned two semesters. This produced a need to cover a great deal of material in a short period of time. Students would typically take just these two courses, BIO 1107-1108, for eight credits towards their biology major over the summer and would not do any other significant activities in that time. Often I received comments from students noting that this was a major body of work to do over the summer, and I would have to agree with that point of view. The extent to which the course worked well was largely due to the skill, effort, and persistence of the students who took these classes. Cornell University benefits from attracting some of the very best students, and this quality of student was shown both in how well many of them mastered the material.

Given this pressure of time, and the amount of material to cover, there were certain things done differently compared to the regular semester courses intended as ways to attempt to help students in their struggle to master the course material. As some of this touches on how a course might be designed, I next will comment on some of these modifications and point out the reasoning behind each.

1) Where possible the material covered in the laboratory exercises had some conceptual material relating to them presented ahead of time in lecture. Often while the same concepts were covered in lecture and lab, different specific examples might have been used. The hope was that this would reinforce student mastery of the core concepts. Thus on looking at the course calendars in the course syllabi it can be seen that often some topics were covered both in lab and lecture. This can also be taken as a choice to have the students cover a bit less material in more depth, rather than to attempt to cover more material broadly. Naturally this meant that there were entire areas of biology that were not covered, and such is often the case in introductory courses.

It may be noted that some instructors of these summer courses when faced with this situation had made the opposite choice, and so had decided that the lecture and laboratory parts of the course would not reinforce each other at all. In that case the lecture and lab had completely different sequences and coverage of topics. The result of that was that students got exposed to much more material, but in a more superficial manner.

2) There was extensive made use of learning objectives in both lecture and laboratory exercises. By telling students what levels of understanding they were expected to be able to achieve it was hoped that their studying could be focused to essential selected issues. Thus the learning objectives were critical in these classes. Each lecture and lab exercise had its own

objectives relating to them. Typically students were given these learning objectives on the first day of class, so that they could then have the option of using them as guides in their reviewing of the assigned readings before our meetings. One other critical practice needed for this objectives based approach to work was that the lecture and lab exams had to have most of the questions in them related in some manner to these learning objectives. This connection gave students incentive to focus on these learning objectives. And with knowledge of the key issues they had to master, often this resulted in students who were very well prepared with topics to discuss during instructor "office-hours".

There are those who object to the use of such explicit learning objectives. Some have presented me with the argument that one of the major abilities students should acquire through taking undergraduate classes is an ability to judge for themselves what are the important concepts and topics covered in a given lecture. From this perspective providing students with explicit learning objectives might be seen as being denied a chance to enhance such an ability. Others have also told me that they consider telling students explicitly what they need to know as little short of "spoon-feeding" them, and that it runs the risk of students focusing in on just a few topics to the exclusion of broader issues.

In response to these views I would say that if the learning objectives are properly crafted this can be an effective means to guide students through complex material. The alternative is to say to students that "everything covered or assigned is fair game on exams" and so risk having some students then spend time considering if they need to be memorizing the content of footnotes in the textbook. I feel that the judgement of what students should spend time studying should be made by the instructor, and conveyed to each student so that it need not be guessed at, and this system of learning objectives was an attempt to meet this need. Anything less, in my view, risks the instructor not having decided what are the important issues covered in a lecture but still presenting material anyway without any attempt to shape the material covered into a larger picture to present to students. The result can be (and sadly I have seen it quite often) a mass of material presented that is not fitted to any larger context, and so the use of explicit learning objectives was a benefit not just to the students but also to myself as the instructor as I struggled to try to craft lectures that illustrated larger issues. Also, in my experience, students of high quality who are asked to do difficult things, and who with guidance and practice meet this expectation, often come out with a sense of being empowered to go on to even more difficult matters. Thus focusing the students' attention onto selected difficult issues was made possible by these explicit learning objectives, and I would recommend the approach be considered in other course settings as well.

3) In these summer courses the letter grades for each course were NOT determined relative to course means, but rather relative to the actually mastery of material displayed in each class. Given that these summer courses were often mostly filled with students who were majoring in biology before applying to medical school (i.e "pre-meds"), and given that typically we had from 20-40 students each summer, we could not use the method for determining letter grades that the regular semester courses used.

The regular semester course had a "curve" to which the mean score for all student work was fitted. Those above the course mean would be assurred of getting a letter grade of B⁻ or better. Those below the mean would be assigned the lower letter grades. This system meant that if a very good student helped a poor student, and so raised the course mean in the process, this

would have the effect of hurting the grade received by the very good student. Obviously this does not make for a "collegial" atmosphere.

So instead in the summer courses we used a different method of determining the course letter grades (see the course syllabi for details). Under this system when students helped explain material to one another, they could also help their own understanding and improve their own mastery of the material, and so achieve a better final course grade. This approach promoted good cooperative student interactions in many cases. While it was still possible for students to receive a low or failing grade, and some did, this system did present the potential, actually realized in a few summers, of every one in the class received course grades in the A and B range. Given the hard work they did in those summers and the mastery they displayed on the course exercises, as an instructor I appreciated having a grading system which did not require me to assign low letter grades to some students merely because they had the bad luck to be in a group of high excellance! Also this permitted me to honestly say to students that they were not competing with one another, but were instead struggling towards their own mastery of the material.

4) Lastly, an issue that is not so much a constraint on the class as set of personal choices. The lecture notes that are given below were made available to students; typically right after each lecture was given they were posted on the course web site. Thus students could see what figures from the text were referenced, and go to specific parts of the text for more information as needed or as directed. Also at the end of each set of lecture notes I often included a list of citations for "related items." These citations were both an attempt to give some mention to a few of the many examples that time would not allow to be fit into a lecture, and were meant to be given as places to look for further information for those wishing to explore such topics later on their own. Obviously none of these articles were required reading, but just having the list there and having some students skim through the titles often gave a nice connection between the introductory material we were covering and published articles in biology. As the instructor, I felt an obligation to my students to share with them in some way a few of the connections I had found that reached out from what we covered in our class... and it gave me a wonderful excuse to read broadly as well!

Scott T. Meissner

Permission to make use of the materials in this file:

4/9/13

To Whom it May Concern:

For many years I was the Visiting Lecturer at Cornell University (Ithaca, New York, USA) in charge of teaching the summer introductory biology courses for biology majors (BIO 1107 and BIO 1108). These courses were last taught in the summer of 2010, after which Cornell University altered its biology majors and eliminated the requirement for a freshman general biology sequence for biology majors. Therefore, these courses are no longer taught at Cornell University.

Enclosed are PDF files from the last time that BIO 1107 and 1108 was taught by me at Cornell. This includes the course syllabi, learning objectives, my lecture notes, the lab manual chapters used, homework sets, and exams that were used.

I give permission to anyone who wishes to use the information from these material as resources for improvement of their lectures, as a source of ideas for lab exercises, etc. All I ask is that when significant items from this source are adopted for use by others fair acknowledgment be made of that fact by them in their classes, lab manuals, etc, as appropriate.

Best wishes to those who take on the challenge of teaching biology broadly!

Dr. Scott T. Meissner
Former Visiting Lecturer, BIO 1107-1108, Cornell University, Ithaca, New York, USA.
Current Visiting Adjunct Faculty member, IBS, CALS, UPLB, The Republic of the Philippines.

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BIO 1107   **Course Information and Policies**   Scott T. Meissner, Lecturer
2010
Welcome to summer Introductory Biology!

The following deals mainly with items relating to the lecture and to general course policies.
Please see the Laboratory Information handout for further information on the laboratory part of
the course.

Course staff and contact information:

		E-mail:
Visiting Lecturer:	Scott T. Meissner	stm4@cornell.edu
Lab Coordinator:	Mark Sarvary	mas245@cornell.edu
Teaching Assistants:	Luis Duque	lod3@cornell.edu
	Corey Ptak	cjp57@cornell.edu
Course Coordinator:	Louise Lattin	lsg6@cornell.edu

The course administrative office is 1140 Comstock Hall. The telephone number is 255-2031.

Texts:

Our text is *Biology*, 8th edition, 2008, by Campbell et al.. New and used copies are
available at the campus bookstore. A copy is available for use in 1122 Comstock Hall.
The BIO 1107 laboratory manual will be handed out in class.

Lectures and Labs:

Lectures will be held from 9:00-12:00 am on Mon., Tues., Thurs., Fridays, and on
Wednesday July 7, in room A106 of Corson-Mudd Hall. At the end of this handout are given a
calendar of lecture topics and a list of reading assignments for each lecture.
Labs will meet from 1:30-4:30 pm Mondays through Thursdays. Each lab will start with
an organizational meeting in room 1116 Comstock Hall. Please see the laboratory general
information handout for more details on the lab part of this course.

Course website:

The course website is: http://blackboard.cornell.edu/. To log on to this site please use
your Cornell Net ID as your username, and use your CU NET password. Once logged in you
should click on the BIO 107-108 link. If you are visiting from another campus, and do not have
a Cornell Net ID, you should visit the Computer Center and get one, and the sign up for a
blackboard account. If you have problems logging in please let me know.
In the website you will find links to answer keys, links to campus policies, copies of
some handouts, etc.... Copies of my lecture notes will be posted, usually within a day after each
lecture. Many documents will be posted as PDF files, which can be read by Acrobat reader.
Please check your e-mail regularly in case you are sent course announcements.

Office hours:

You may come to my office hours to do several things: Ask me questions about course material, describe items to me to get feedback on your mastery of a topic, or just bring a bag lunch and sit in a soft seat before going to lab in the afternoon... No appointment needed.

I will hold office hours at the following times in room 1122 Comstock Hall:
12:25-1:25, Monday through Friday, and also
10:00-12:00, Wednesday mornings.

Other times may be possible by appointment, especially earlier Wednesday mornings or later Friday afternoons. Questions sent by e-mail will be joyfully received and answered when I can. I am happy to discuss items from lecture, or lab, as you see fit.

Points and assigning letter grades:

You will receive one letter grade for BIO 1107, which will include points from lecture and from lab. The letter grades for BIO 1107 will be set relative to the highest number of points **actually earned** by someone in the course. As a rough guideline: Anyone who earns 90-100% of that highest earned score will be in the "A" range (A^+, A, A^-), 80-90% of the highest score will earn a grade in the "B" range (B^+, B, B^-), 70-80% of the highest score will be the "C" range, 60-70% of the highest score will be the "D" range, and below 60% of the highest earned score will be failing. I reserve the option of considering such additional issues as attendance, improvement in the course, participation, and other factors when determining course grades.

There will be 300 total points for lecture and 300 total points for lab. For a total of 600 points. Here is the breakdown of the points that will be assigned for BIO 1107:

Lecture	Exam 1	70 points
	Exam 2	70 points
	Exam 3	145 points
	Three Homework Sets (5 points each)	15 points
	Lecture Total:	300 points
Laboratory	Quiz 1	35 points
	Quiz 2	35 points
	Descriptive Statistics Worksheet	14 points
	Statistics II Worksheet	14 points
	Photosynthesis Worksheet	14 points
	Fetal Pig Worksheet	14 points
	Biomechanics Worksheet	11 points
	First Submission Poster Text	15 points
	Final Submission of Poster	25 points
	Poster evaluation	9 points
	Individually written abstract	14 points
	Practical Exam	100 points
	Laboratory Total:	300 points
	Total for entire course:	600 points total

Exams:

There will be three lecture exams for BIO 1107. Lecture exams will cover material from lecture only, and will not explicitly cover items raised in lab part of the course. The following is the coverage for each exam:

Exam 1: 9:00-11:00 am, Tuesday June 22;   Covering material from lectures 1-11.
Exam 2: 9:00-11:00 am, Thursday July 1;   Covering material from lectures 12-23.
Exam 3: 9:00-12:00 am, Friday July 9;   Covering material from all lectures.

Homework sets:

The purpose of these homework sets is to give you practice with some of the material before confronting it on exams. Be aware that since these homework sets are "open book" some of the questions in them go into more detail than I would expect of you on exams. The intent is for you to get feedback on the material before each lecture exam, but since it may take me a while to grade these sets, I suggest that you keep a photocopy or scan of your work so that you can immediately compare your answers to the posted answers before each exam.

The first two homework sets will be due at or before the start of lecture (9:00 am) on their due dates. The third homework set is due by 1:00 pm on Wednesday July 1, when I will be in 1122 Comstock Hall.

Homework set 1:   Covers lectures 1-11.   Due: 9:00 am, Monday, June 21.
Homework set 2:   Covers lectures 12-23.   Due: 9:00 am, Tuesday, June 29.
Homework set 3:   Covers lectures 24-31.   Due: 9:00 am, Wednesday, July 7.

Homework sets handed in late will suffer a loss of two points out of the five that each homework set is worth. No homework set will be accepted for grade if turned in after its answer key is posted, which will typically be by noon on the day it is due.

Academic integrity and acknowledging the work of others:

As pre-professionals you are encouraged to discuss the material covered in this course with one another. However, the answers you give on exams, homework sets, or other assignments in this course are expected to be your own work, given in your own words.

For the sake of homework sets it is assumed that the source of facts used in answers is either the textbook or the lecturer, and for such cases no citing of a source of facts is needed. But in cases where you choose to make use of the writing of others (whether actual words or the flow of ideas, or to make use of an apt phrase, to paraphrase, or to quote from any source), you are expected to clearly indicate what part(s) of an answer are your writing and what part(s) are from another source. In such cases you are required to both cite the source appropriately and use quotation marks as needed. Failure to do so may result in a loss of points on an assignment, or in extreme or repeated cases lead to a charge of academic misconduct.

The Writing Appendix of the lab manual has information concerning how to appropriately cite sources, and information about relevant policies of Cornell can be found in the publication The Code of Academic Integrity and Acknowledging the Work of Others (available in the course web site and at: http://web.cornell.edu/UniversityFaculty/docs/AI.Acknow.pdf).

Answer keys and requests for regrades:

Suggested exam and homework answers will be posted in the display case in the hallway leading to room 1122 Comstock Hall, and on the course website, after each exam or due date. In many cases questions can have more than one correct answer, and for many questions partial credit may be earned to some extent. You may (indeed I hope you will!) wish to discuss the questions and answers with me after taking exams.

If you wish to request that the grading of a question used in lecture be reconsidered please note the following: I suggest that you first check the posted answer keys and the text, and then come and talk with me about any items before you request a formal regrade of an item. Then, for most lecture items (except for the final exam) you will have until noon on the last day of the course (July 9) to submit written arguments requesting regrades. Each request should be accompanied by the original graded item and a description of your reasons for reconsideration. For exam #3 you will have until noon of July 10 to e-mail me comments about possible alternate answers. I will try to be generous when reconsidering items, but you can help by presenting me with tightly reasoned arguments pointing out either an error in grading, or an alternate answer for a question, and by supporting your arguments where possible by references to our text or other sources.

Please see the lab coordinator concerning regrading of any lab-related materials.

Absences and last day to withdraw:

Attendance at lecture and laboratory is required. I am required to document attendance, so please check your name on the lecture attendance sheet each day. This is an accelerated course with material being covered very rapidly. Thus each day of lecture and laboratory in this course is equivalent to a week's worth of material from a course in the regular semester. Be aware that no make-up exams are planned. If for any reason you will not be able to attend class or if you fall ill, or if something else prevents you from doing the work for this course, please notify me as soon as possible. The last day you can arrange to withdraw with a full refund of tuition is the third day of the course (June 16).

How I suggest you approach the lecture material of this course:

Each day of lecture will usually run for three hours. Each hour of lecture has reading assignments which often comprise a chapter of the textbook. And then there is the lab material. You should expect to spend evenings and weekends focusing on the material from this class. Here is some advice for approaching the lecture material of this course.

For each lecture I will give you a set of learning objectives. My goal is for the majority of the questions on the exams to have some connection to those objectives. But there may be some questions that may not be explicitly stated in the objectives.

I view the text book readings as mainly supplementary and as another means for you to get information on critical items raised in lecture. Use the book mainly as a means to expand on the concepts raised in lecture, to review the definitions of key terms, or to review important structures and functions, as well as to explore new examples and implications of those concepts. In some of the lectures' learning objectives I have noted questions at the end of chapters in the text that I feel might be useful to you, be sure to try them and see the appendix in the back of the text for the answers. Use the learning objectives and the items covered in lecture as a guide to focus your attention as you read. This means that you should indeed read the assigned reading, but do not try to learn everything in the text. Rather you should attempt to master all the items raised in each lecture's learning objectives, and be able to consider implications of that material.

Here is what I suggest that you do before coming to each lecture: Review the learning objectives for that lecture and get a sense of what sort of topics you will be expected to master. You might want to jot down a list of topics or concepts that you identify as key ones, just to fix them in your mind a bit better. Then skim through the assigned readings and pay particular attention to areas that you can identify that relate to the lecture's objectives. Each chapter of the text has a good summary section at its end and some people find this helpful.

Then come prepared to listen and take notes at lecture. This means being awake and taking good active notes. Don't fall into the trap of trying to write down each and every word, but do attempt to synthesize and summarize key points as you identify them.

After lecture: Review your notes and read the text more deeply to get another point of view on the material. My posted lecture notes include a list of all the figures from the text that I use in each lecture. So you should be able to use them to help you focus in on the parts of the text that are most relevant. You should run through the learning objectives several times, daily is ideal, and quiz yourself on important terms, concepts, structures and processes. Identify topics that may need more attention and spend some time working on them. Review those specific topics by reviewing sections of the text, or by referring to my posted lecture notes. Starting to work on the question(s) in the homework set that relate to that day's lectures is another good thing to do.

As you do this cycle of studying and reviewing you may come up with questions that you may wish to ask me. Please do contact me as you feel the need. If you have a burning issue at midnight you can send me an e-mail, but be aware that I will most likely be asleep and will not read it until the next morning. But I will most likely be able to send you a reply before lecture. Another approach is for you to note your question and bring it to my office hours the next day. In the past I have been happy to have many people who took this course use both of these approaches, and there have been many students with whom I had multiple contacts each week.

Most importantly, keep plugging away at the material, and stay in touch with me and the laboratory staff via e-mail or at office hours. In the past some good hard work has seen many students through this course and on to a good outcome, and hopefully this will be the case for you as well!

I look forward to working with you!   STM

Lecture reading assignments for BIO 1107 Lectures:

Date:	Lecture #	Topics:	Readings: (Campbell et al., 2008)
June 14	1)	Introduction to the Scientific Method	Course Information and Policies Handout, text pgs. 18-24, and chapter 1 of the Lab Manual.
	2)	Life and Evolution	Pgs. 1-17.
	3)	Carbon Chemistry	Chap. 2, 4 (skim chap. 3).
June 15	4)	Classes of Organic Matter	Chap. 5.
	5)	Enzymes	Chap. 8.
	6)	Cell and their parts	Chap. 6.
June 17	7)	Membrane Structure and Function	Chapter 7 (but ignore pgs. 133-134), and see pgs. 768-770, 954-955.
	8)	Respiration I	Pgs. 162-172, 177-182.
		Scientific writing	Lab Manual Writing Appendix, and pgs. 16-22 of The Code of Academic Integrity and Acknowledging the Work of Others (http://web.cornell.edu/UniversityFaculty/docs/AI.Acknow.pdf).
June 18	9)	Respiration II	Chap. 9.
	10)	Photosynthesis	Chap. 10.
	11)	Signal Reception, Transduction, Response	Chap. 11.
June 21	12)	Eukaryotic Diversity I: Protista	Chap. 28, and pgs. 538-539, 542-544, 551-553
	13)	Being Multicellular	Chap. 40, and pgs. 118-121, 517-518, 565.

Date:	Lecture #	Topics:	Readings: (Campbell et al., 2008)
June 22	14)	Digestion	Chap. 41.
June 24	15)	Transport I	Pgs. 898-915.
	16)	Gas Exchange	Pgs. 776-779, 915-927.
	17)	Plant Nutrition	Chap. 37.
June 25	18)	Excretion	Chap. 44.
	19)	Movement I	Pgs. 112-118, 558-559, 838, 842-843, 1105-1114.
	20)	Plant Dispersal	Pgs. 804-805, 809-811.
June 28	21)	Hormones	Pgs. 207-210 and chap. 45.
	22)	Animal Reproduction	Chap. 46.
	23)	Transport II	Chap. 36.
June 29	24)	Eukaryotic Diversity II: Chordates and Vertebrates	Pgs. 445-446, 513, 525-528, 654-655, 661-663, chapter 34, and Appendix E.
	25)	Movement II	Pgs. 1112-1117, fig. 40.5 (connective and muscle tissues), and the chapter on Biomechanics in the BIO 1107 Lab Manual.

Date:	Lecture #	Topics:	Readings: (Campbell et al., 2008)
July 1	26)	Study of Molecular Diversity	Pgs. 97, 405 (fig. 20.9).
July 2	27)	Action Potentials	Pgs. 1047-1056.
	28)	Synapses	Pgs. 120-121, 1056-1061, 1078-1080.
	29)	Nervous Systems	Pgs. 1048, 1064-1078, 1080-1084.
July 6	30)	Senses I	Pgs. 1087-1105.
	31)	Senses II	Pgs. 1087-1092, 1094-1096, 1099-1100.
	32)	Comparative Physiology	Pgs. 708-723, 862-872, 881, 891-893, 900-903, 917-918, 920-921, 925-927, 954-960, 968-969, 1000-1001.
July 7	33)	Animal Behavior	Chap. 51.
	34)	Non-Animal Behavior	Pgs. 207, 777-778, 821-824, 838, 842-843.

BIOLOGICAL SCIENCES 1107 [14 JUNE - 9 JULY, 2010]

	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
Lecture Week 1	14 June 1) Intro, Sci Meth. 2) Evol. and Life 3) Carbon Chem.	15 June 4) Organic Matter 5) Enzymes 6) Cells	16 June Office hours	17 June 7) Membranes 8) Respiration I Lab: Scientific Writing	18 June 9) Respiration II 10) Photosynthesis 11) Signal Transduction
Laboratory Week 1	Field Trip	Microscopy	Enzymes	Osmosis **Desc. Stat. Wkst. Due**	
Lecture Week 2	21 June **(Homework 1 Due)** 12) Eukarya Diversity I: Protista 13) Multicellular (and review)	22 June **Exam I** 14) Digestion	23 June Office hours	24 June 15) Transport I 16) Gas Exchange 17) Plant Nutrition	25 June 18) Excretion 19) Movement I 20) Plant Dispersal
Laboratory Week 2	Photosynthesis **Stat. II. Wkst. Due**	Protista	Fetal Pig I **Quiz #1**	Fetal Pig II **Photosynthesis Wkst. Due**	**Poster Text due at 9:00 am**
Lecture Week 3	28 June 21) Hormones 22) Animal Reproduction 23) Transport II	29 June **(Homework 2 Due)** 24) Eukarya Diversity II 25) Movement II (and review)	30 June Office hours	1 July **Exam II** 26) Study of Molecular Diversity	2 July 27) Act. Pot. 28) Synapses 29) Nervous Systems
Laboratory Week 3	Angiosperm Structure and Function **Fetal Pig Wkst. Due**	Biomechanics I	Biomechanics II **Quiz #2**	Plant Pigments	**Biomech. Wkst. Due at 9:00 am**
Lecture Week 4	5 July (No Class)	6 July 30) Senses I 31) Senses II 32) Comparative Physiology	7 July **(Homework 3 Due)** 33) Animal Behavior 34) Non-Animal Behaviors	8 July Office hours	9 July **Final Exam**
Laboratory Week 4	(No Class)	Lab review	**Poster and Abstract Due** **Lab Practical Exam**		

BIO 107   2010
Day 1, Lecture 1, Scientific Method..
Text Readings: Course Information and Policies Handout; Campbell et al. (2008), pgs. 18-24;
Lab Manual chapter 1.

Objectives:

  Know the stages of the scientific method. What should a good scientific model be able
to do? Be able to contrast inductive and deductive reasoning. How do observational studies
differ from experimental studies? Which is more likely to have a control group, and what is the
purpose of a control group? Just because a model has been produced and has evidence to
support it does that make it valid in all cases? Does Kettlewell's model of the affects of
predation on moths apply to all species of moths, or does it have specific limits?

  Be able to describe and contrast the uses of null and alternative hypotheses. Explain the
purpose of these statements in the scientific method: Which gives the most specific prediction?
Given an example of an experiment be able to state null and alternative hypothesis that are
appropriate for that specific experiment. Understand the difficulties of "proving" a hypothesis
compared to the relative ease of disproving a hypothesis. Can you relate this to why we use the
null and alternative hypotheses as a means to clarify our thinking?

  Given an example of part of a scientific investigation be able to describe the aspects of
the scientific method it contains. For instance, given the description in the lab manual you
should be able to describe how Kettlewell used various stages of the scientific method at
various points in his studies.

BIO 107   2010
Day 1, Lecture 2, Title: Life and Evolution.
Text Readings: Campbell et al. (2008), pgs. 1-17.

Objectives:

  Be able to describe several structural features of life, and several functional features of
life, as found on Earth. Describe how reproduction differs from replication. If given a draft
definition of life be able to critique it in terms of what important features of life it includes and
propose several features that it does not cover.

  Be able to identify five essential preconditions for Darwinian evolution to occur.
Characterize biological evolution in terms of: The time scale over which it occurs. The ability
of individual organisms to undergo it. A likely mechanism that drives it.

  Contrast the meaning of the term "evolution" as used in the context of the biological
theory of evolution versus its meaning in common speech.

BIO 107   2010
Day 1, Lecture 3, Title: Carbon chemistry.
Text Readings: Campbell et al. (2008), Chapters 2 and 4 (and skim Chapter 3).

Objectives:

Be able to relate atomic properties, especially in terms of the number of bonds and polarity of bonds that can be made, to the properties of molecules made up of those atoms. Be able to compare covalent bonds, ionic bonds, H-bonds, and Van der Waals interactions in terms of their relative strengths and properties.

Be able to order carbon groups in terms of their reduction states. What are some ways in which carbon is an ideal atomic base for organic matter? Consider the influence of electronegativity, valence, and the asymmetric carbons on the variation of the properties of molecules that can be produced.

Be able to describe and recognize different bond types that exist in organic molecules and between molecules. If given any two molecules (for instance pick any two shown in figure 5.17 on pg. 79 of our text) be able to identify all the ways in which they might interact. Or if given any one of these molecules you should be able to identify polar and non-polar bonds, functional groups, acids, bases, and asymmetric carbons in it. By examining a molecule be able to determine if it is very water soluble or not.

(We do not have time to go into the material in chapter three of the text. But you might wish to skim over the material and be sure you have a general understanding of the following items covered in it as useful background on water: Cohesion, role of water as a solvent, its ability to H-bond, and pH.)

Suggested review questions. Chapter 4: # 3, 4, and 5.

BIO 107   2010
Day 2, Lecture 4, Title: Classes of Organic Matter.
Text Readings: Campbell et al. (2008), Chap. 5.

Objectives:

Be able to recognize and describe examples of condensation reactions. How do they differ from hydrolysis reactions? Are these condensation reactions a type of redox reaction, why or why not?

Be able to discuss the major characteristics of the four major classes of organic molecules, and the polymers and monomers of three of them. Be able to describe why lipids are not considered to have polymeric forms.

If shown the molecules given in figures 5.5, 5.13, 5.17, or 5.27 be able to recognize hydrophobic or hydrophilic, charged or uncharged, regions of the molecules shown. For those that are monomers, be able to describe what parts of them are used in polymerizations.

Be able to recognize and discuss the role that hydrogen bonding, van der Waals interactions, disulfide bonds, and ionic bonds each play in molecular folding and interactions. Be able to identify bonds involved in determining polymer shapes that are weak, and others that are strong, and relate each to the polymer's functions.

Be able to recognize the difference(s) between items such as those listed below, and know for which class(es) of organic matter each is applicable:
>   RNA and DNA?
>   A phospholipid and cholesterol?
>   Hexoses and pentoses?
>   Primary, secondary, tertiary and quaternary structure?
>   An alpha-helix and a beta-sheet?
>   A purine and a pyrimidine?
>   A diacylglycerol and a triacylglycerol?
>   A monosaccharide and a disaccharide?
>   Ester bond, phosphoester bond, glycosidic bond, and a peptide bond.
>   Water soluble and water insoluble molecules.

The "self-quiz" questions #1, 2, 6, and 8 at the end of chapter 5 are worth a look.

BIO 107   2010
Day 2, Lecture 5, Title: Enzymes and Metabolism.
Text Readings: Campbell et al. (2008), Chapter 8.

Objectives:

What ultimately determines the rate of a reaction? What determines the net direction of a reaction? What determines whether or not a molecule is relatively stable, compared to whether or not a reaction is spontaneous? Be able to describe the change in free energy as a set of molecules are taken through a reaction cycle to become a set of products when: The reaction is endergonic? The reaction is exergonic?. The reaction is enzyme catalyzed compared to when the reaction is not enzyme catalyzed? The temperature or pressure of the system is raised, compared to the state at a lower temperature or pressure?

(Please note: The text makes references to "high energy molecules" and to the storage of energy "in" bonds or in molecules. I feel it is better to compare energy differences between two states rather than to attribute the energy to one state. Thus, in my view ATP is not a "high energy" molecule with energy stored "in" it, rather the hydrolysis of ATP represents a net release of energy as the energy state of the products is lower compared to that of the reactants. So when you read the text be careful not to fall into the trap of thinking that energy is stored in bonds.)

What are ways that an enzyme can interact with its substrate? Given a specific amino acid and substrate be able to describe several types of bonds, if any, that might be formed between them.

What are two properties a specific enzyme might have that could be used to isolate it from all the other organic molecules in a tissue?

What are V_m and K_m? What information does knowing each of these give you? What are competitive inhibitors and non-competitive inhibitors and how do they differ? What is allosteric regulation of an enzyme's activity, what is needed for this to occur, and how does it differ from covalent modification of an enzyme?

How do enzymes couple reactions? What is the advantage of coupling reactions through the use of enzymes? Be able to describe examples of how an enzyme's ability to couple reactions are be used by cells to convert one form of energy into another form of energy.

Be able to describe several ways in which an enzyme's activity can be altered in a cell.

In chapter 8 of the text, self-quiz questions 4 and 5 are nice.

BIO 107   2010
Day 2, Lecture 6, Title: Cells and their parts.
Text Readings: Campbell et al. (2008), Chapter 6.

Objectives:

Contrast the structures of a typical bacterial cell versus that of a typical eukaryotic cell. Be able to describe the structure and major function(s) of each cellular part (see figures 6.6 and 6.9 for those that are fair game). What are two differences between plant and animal cells? What are the typical differences between prokaryote and eukaryote cells?

What is an advantage and a disadvantage of a large surface area relative to internal volume? What traits are likely to be seen in a larger or more metabolically active cell? Be able to describe features of eukaryotes cells that act to help the cells overcome the limits of diffusion.

Which eukaryotic organelles contain DNA? How did they get their DNA and how does this relate to the number of membranes each has? What is endosymbiosis? Are there compartments in the cell that lack ribonucleic acids, if so which compartments are they? Which organelles are part of the endomembrane system? Describe the typical flow of membrane; where in the cell is it made and where does it go on its way to the plasma membrane? What is a common feature of the aqueous space within the endomembrane system?

What are several ways in which cells may attach and form junctions with other cells? Which are often used by animal cells and which are often used by plants? What is the purpose of each type of junction? Consider how the use of cell-to-cell connections allows for the organization of cells into tissues with distinct functions.

What is the cytoskeleton? Is it found in prokaryotes or in eukaryotes? Know the major proteins that are a part of the cytoskeleton, the structures they make, and the functions with which they are often associated. What are motor proteins and what do they do?

The extracellular matrix varies broadly. Describe major differences in this as seen in bacteria, animal, plant and fungal cells.

Self-quiz questions #1, 2, 3, 5, 7 at the end of chapter 6 are worth a look.

BIO 107   2010
Day 3, Lecture 7, Title: Membrane Structure and Function.
Text Readings: Campbell et al. (2008), Chapter 7 (but ignore pgs. 133-134), and see pgs. 768-770, 954-955.

Objectives:

Be able to describe the important structural and functional aspects of the Singer-Nicholson Fluid-Mosaic model. How do integral membrane proteins differ from peripheral membrane proteins? What must those proteins that take part in a mosaic be doing that the fluid proteins do not?

What are types of energy normally converted by organisms from one energy form to another? What class of molecules is typically used by cells to do this conversion? Be able to give examples of such energy conversions.

Define osmosis and given sufficient information be able to determine which way water will flow across a membrane. Energy in what forms often influences the movement of water across biological membranes? Be able to describe two solutions in terms of their relative osmolarity (i.e. hyperosmotic, hypoosmotic, or isoomotic). Describe how even though a cell is hyperosmotic to surrounding pond water (i.e. the pond water is hypoosmotic relative to the cell's contents), yet the water in the cell and the pond can be at the same energy level.

How are transport systems able to move items into the cell up their concentration gradient?   What properties of molecules influence their ability to cross a biological membrane? How do ion channels differ from ion pumps? What is the difference between active and passive transport? Be able to describe examples of both types of transport. Describe an example of a co-transport system. How do symport co-transport systems differ from antiport co-transport systems?

What are some other functions that membrane proteins address for cells other than that of physical transport of items across the membrane?

Self-quiz questions #2 and 5 of chapter 7 are worth looking at.

BIO 107   2010
Day 3, Lecture 8, Title: Respiration I.
Text Readings: Campbell et al. (2008), Chapter 9, pgs. 162-172, 177-182.

Objectives:

Be able to identify the starting and ending points of: glycolysis, fermentation, pyruvate import, and the citric acid cycle. For each of these processes be able to describe the pathway in terms of substrates consumed and products produced and the final net result. Know where and under what conditions each is typically done in the cell. Be able to rank different reactions in terms of their relative energy changes.

What are redox reactions? Given two carbon compounds be able to identify which is more oxidized and which is more reduced by examining the redox state of each of their carbons. If shown the molecules involved be able to identify whether a reaction is or is not a redox reaction. When given sufficient information about a reaction be able to determine whether or not it is a spontaneous reaction, and whether or not the reactants are stable under the stated reaction conditions.

Phosphate bonds play an important role in energy transduction in cells. Consider examples from the intermediates in glycolysis that would be able to promote the formation of ATP and those that would not. What is substrate-level phosphorylation? If shown the molecules involved in one reaction of the respiratory pathways be able to identify whether it is directly involved in substrate-level phosphorylation.

When does a cell shift to fermentative respiration? With what problems does fermentation deal and what benefits does it provide?

What compartments of the cell are involved in the various stages of cellular respiration? How does compartmentation of biochemical pathways benefit eukaryotes? Where in an aerobic bacterium would these pathways be carried out?

Understand how different sources of carbon molecules from various types of food can be funneled into glycolysis.

What are the ways in which feedback can play a role in regulating cellular respiration? At what point(s) in the process does this regulation typically take place?

Self-quiz questions #1, 3, and 9 of chapter nine are worth a look.

BIO 107   2010
Day 4, Lecture 9, Title: Respiration II.
Text Readings: Campbell et al. (2008), Chapter 9.

Objectives:

What is chemiosmosis, and what eukaryotic cellular compartment(s) does it involve? What does it create in mitochondria, and what must be present in order for it to take place? What are the substrates and products of chemiosmosis and what enzyme does it use? What is the electron transport chain, and what cellular compartment(s) does it use? Be able to describe the substrates and products of each of the electron transport chain's three major membrane spanning complexes. In the course of oxidative respiration what forms of energy does the cell transduce from beginning to end of all of oxidative respiration?

More ATP are formed with the oxidation of NADH found in the mitochondrial matrix than are formed with the oxidation of $FADH_2$ found there, and also it is more than are formed by the oxidation of cytosolic NADH. Why is this? For each NADH oxidized in the matrix where are protons moved to, and why does their destination matter?

Where in the process of oxidative respiration is oxygen gas consumed?

Know the parts of mitochondria, and the areas of the cell in which each of the major stages of oxidative respiration occurs. Be able to relate the parts of the mitochondria that are derived from corresponding parts of a free living bacterium.

If a compound was added to the mitochondria that could accept electrons from reduced cytochrome c so that it did not pass any electrons further down the electron transport chain what effect would this have on: The proton concentration gradient across the inner mitochondrial membrane? The consumption of oxygen? The rate of glycolysis? The regeneration of NAD^+ in the mitochondrial matrix? The rate of carbon dioxide production? The rate of ATP production?

For one glucose respired in the presence of oxygen what would be the number of ATPs that would be expected to be produced? What if you started with just one pyruvate, or one NADH found in the mitochondrial matrix? What amount of ATP would be expected to be made if the cell lacked oxygen?

For review, see self-quiz questions #2, 4, 5 and 6 of chapter 9.

BIO 107   2010
Day 4, Lecture 10, Title: Photosynthesis.
Text Readings: Campbell et al. (2008), Chapter 10.

Objectives:

In what ways are the photosynthetic processes done in the chloroplast similar to respiratory processes done in the mitochondria? Be able to compare the compartments, pathways, and redox states of carbon of these two processes. Be able to compare how chemiosmosis is used in photosynthesis and respiration.

Be able to describe for the light and dark reactions of photosynthesis: The substrates and products of each and compartments of the chloroplast involved. What role does water, NADPH, ATP and the thylakoid membranes play in photosynthesis? In carrying out photosynthesis what forms of energy are transduced? In the light reactions, and later in the dark reactions, what exergonic reactions are used to power endergonic steps? What type of carbon compound does the chloroplast normally export to the rest of the cell as a result of photosynthesis? What compound does a cell in a leaf normally export to the rest of the plant?

RUBISCO is a major enzyme in the Calvin cycle. What reaction does it catalyze and what are all its possible substrates? How does photorespiration complicate the fixation of carbon into organic matter by the plant? Be able to determine whether either of these reactions (carbon fixation and photorespiration) are redox reactions.

During photosynthesis what is happening to the redox state of carbon? What is needed for these changes to be accomplished? In the chloroplast what is the ultimate source of the electrons and hydrogen ions that are used to reduce the carbons?

Describe the path that carbon dioxide typically takes through a leaf as it goes from outside a plant up to its fixation in a chloroplast. Be able to describe the carbon dioxide concentrating mechanisms used by C4 and CAM plants. How does each modify the activity of their dark reactions in terms of space and time? What does this mechanism do to the plant's ability to restrict water loss?

For chapter 10 see self-quiz questions #1, 3, 4, 5 and 7 for review.

BIO 107   2010
Day 4, Lecture 11, Title: Signal Reception, Transduction, Response.
Text Readings: Campbell et al. (2008), Chapter 11.

Objectives:

What models do we have to explain how information indicating the presence of external signals gets transmitted across the plasma membrane and results in changes in cellular behavior or growth?

What difference does it make if the signal-molecule is hydrophobic or hydrophilic in terms of where the cell may have a receptor for it? Be able to describe what typically happens at the molecular level during signal reception. Be able to describe the three major classes of receptors we covered. Which of these three types of receptors is most likely to be fastest in affect?

Be able to describe the bacterial two-component system as an example of prokaryotic signal reception and transduction, and compare it to the eukaryotic receptors we cover.

What options do cells have for internal signal transduction? Be able to describe examples in which the cell modifies existing proteins as well as how the cell might alter the concentration of secondary messengers in a cellular compartment. What is one consequence of having many transduction steps in series between the reception and the response to a signal in terms of the speed of the system? What must occur for this arrangement to be adaptive and so be selected for through evolutionary processes?

What is one consequence of different signals activating different receptors that then activate transduction using the same secondary messenger in the same cell? Will this always result in a common response to different signals? How can a rise in calcium ion concentration in a muscle cell's cytosol cause one type of response, while a rise in calcium ion concentration in a nerve cell's cytosol causes a different type of response?

After responding to a signal what does the cell have to do to turn off the response and reset the transduction system? Given an example of a signal transduction system (for instance, see figure 11.13) be able to describe what will have to be done to reset the system. What must be done to each of the three receptor classes after use to reset them for subsequent signal reception?

Self quiz questions #2, 3, 6 and 7 of chapter 11 are worth a look.

BIO 107   2010
Day 5, Lecture 12, Title: Eukaryotic Diversity I: Protists.
Text Readings: Campbell et al. (2008), Chapter 28, and pgs. 538-539, 542-544, 551-553.

Objectives:

What are examples of characteristics shared by humans and unicellular protists? Consider how a study of these protists can be used to explore the nature of cells in humans.

What is the distinction between primary endosymbiosis and secondary endosymbiosis? What are examples of organisms which are thought to have come about by each type of endosymbiosis?

How is paramecial sex different from our own? What role does the macronucleus and the micronuclei play in their lives?

Be able to describe how you could determine whether endosymbiosis has occurred and whether it is primary or secondary endosymbiosis.

How has the existence of various types of endosymbiosis complicated attempts to classify organisms according to their evolutionary history? Be able to distinguish vertical versus horizontal gene flow and describe how knowing the past occurrences of such events is important for attempts to reconstruct past evolution.

Given a hypothetical cladogram be able to discern the relationships shown in it and the implied order of appearance of traits it shows.

Know general traits of the following groups [*Euglena* sp., *Paramecium* sp., *Pfiesteria* sp., *Dictyostelium* sp. (Slime mold), Diatoms, *Chlamydomonas* sp., Kelp]. Some important traits to consider include: The presence and nature of any cell walls. Coenocytic species. The presence of macro- and micro-nuclei. Whether that group is autotrophic or heterotrophic. Specific means of locomotion. Be able to arrange these groups using their traits in a hypothetical phylogenetic tree showing which traits appear at the various branch points.

For review see self-quiz questions #2 and 4 of chapter 28, and #4 of chapter 26.

BIO 107   2010
Day 5, Lecture 13, Title: Being Multicellular.
Text Readings: Campbell et al., (2008), chapter 40, and pgs. 118-121, 517-518, 565.

Objectives:

Be able to describe several major challenges that organisms must meet if they are to be viable multicellular organisms that they did not face as single celled organisms. What are some solutions that life has evolved that meet these challenges? Look for characteristics of single-celled organisms that could be used to meet some of the challenges of a multi-celled organism. What are some traits found in prokaryotes, and what are others found in single-celled eukaryotes, that can be used by multicelled organisms to address their needs? What are some things that multicelled organisms have to worry about that most single celled organisms do not, and what are some benefits multicelled organisms gain in being multicellular?

Describe factors that can alter the rate of diffusion of an item. What are several ways that an organism can modify itself or its surroundings to enhance the total amount of items that diffuse up to its cells? Conversely how might it make a better barrier so that it can limit loses due by diffusion?

Be able to describe homeostasis in animals. What would be an example of homeostasis in a non-animal species? What are some cellular and molecular mechanisms that must be present for homeostasis to be accomplished? What is a negative feedback system, and what is a counter current exchange system? Be able to describe examples of the uses of these in an animal's temperature regulation. Be able to describe various examples of types of sensing, signal transduction, and responses that are often used as an animal carries out its temperature homeostasis.

Be able to describe the major tissues found in animals and their general functional roles. Relate them to how they help the organism meet challenges unique to being multicellular. What must be present to have an animal tissue?

Self-quiz questions #1, 3, and 6 of chapter 40 might be helpful for review.

BIO 107   2010
Day 6, Lecture 14, Title: Digestion.
Text Readings: Campbell et al. (2008), Chapter 41.

Objectives:

Be able to define digestion. Compare how and where digestion is carried out by bacteria, unicelled protists, fungi, and animals. In animals, how does digestion differ in an animal with a gastrovascular cavity (i.e. hydra) versus in an animal with a complete digestive tract (i.e. humans). How does digestion assist these organisms to over come problems related to diffusion? Where must the items diffuse to and what must happen to them once they get there? In what ways (if any) do these organisms attempt to alter things so as enhance the rates of diffusion of desired items to themselves?

What types of organic matter are absorbed, and what fundamentally limits the rates of absorption? What is the state of the organic matter when it is first consumed, and how is it altered before it is absorbed? Be able to describe the major groups of enzymes involved with digestion of each of the major types of organic matter, and know their origins in the mammalian system.

Know the major organs and their functions in the mammalian digestive tract. What are the specializations of each? Which are exocrine glands and what are the functions of items they secrete?   Be able to describe the major functions of each organ in terms of how major types of organic matter consumed is prepared for absorption. How would absorption of a hydrophilic item most likely differ from that of a hydrophobic item?

In what ways does diet correlate with characteristics of a species' digestive tract? For example, how might a herbivore's digestive tract be expected to differ to from that of a carnivore's?

When an organism makes digestive enzymes how does it avoid digesting itself? What are several options an organism has which allow it to avoid this problem? Be able to describe uses of compartments in this regard, either within one cell, or within one organism. Is digestion ever carried out across several organisms?

For review, see self-quiz questions #4-8 of chapter 41.

BIO 107   2010
Day 7, Lecture 15, Title: Transport I.
Text Readings: Campbell et al. (2008), pgs. 898-915.

Objectives:

What is the hepatic portal system, where does it occur, and what role does it play? What is a "portal system"? What part of a fish's circulatory system is a portal system?

Be able to trace the flow of blood through the mammalian heart out to the rest of the body and back again, identifying the major chambers of the heart and immediate veins and arteries connected to it (see figures 42.6 and 42.7 for the level of structures to consider here). How is the heart beat sequence regulated? Be able to describe the role of the nodes.

How do veins differ from arteries? What changes happen to blood pressure and velocity of flow as blood travels away from and back to the heart? Given the limits of diffusion how close must a cell be to a capillary bed?

Blood flow in circulatory beds and accompanying fluid loss is an important issue. What causes the fluid loss? How is fluid that is lost from the blood recovered? Are all capillary beds always open, if not how is the flow through them altered?

What are ways in which blood flow in the human fetus changes at birth to give the adult pattern of circulation? What structures are involved in these changes and what are the reasons for these structures in the human fetus in terms of the flow of blood?

Fish have a two chambered heart. What is the consequence of this on their blood pressure? Frogs breath through either their lungs or their skin, in what way does a three chambered heart make sense in this case? What would allow an animal to have an open circulatory system, and what traits might force an animal to have a closed circulatory system? What are examples of animals for each of these two types of circulation? What is an example of an animal that lacks an internal circulatory system and how does it manage to live without one?

Be able to describe the functional purposes of a circulatory system, and consider the types of energy transductions an organism must carry out to make it work.

For review see self-quiz questions #2 and 3 of chapter 42.

BIO 107   2010
Day 7, Lecture 16, Title: Gas Exchange.
Text Readings: Campbell et al. (2008), pgs. 776-779, 915-927.

Objectives:

Plants, fungi, and animals such as planaria, earthworms, insects, fish, birds and humans all must deal with the exchange of gases. Be able to compare how each of them achieves this essential goal. What difference does the medium make through which they attempt to carry out gas exchange?

Be able to describe how plants open and close their stomata to control gas exchange. What gases move through their stomatal pores, and typically each moves in which direction?

What are the advantages and disadvantages of breathing through skin versus breathing through a specialized gas exchange structure such as a lung, a tracheal opening, a stomatal pore, or a gill? What structural aspects of the alveoli improves its effectiveness for gas exchange? Be able to point to similar aspects in gills and trachea and in leaves.

How does counter current exchange increase the efficiency of gas exchange? What are examples of organisms that have counter current gas exchange systems and others that do not? What items, other than gases, could have their exchanges enhanced by a counter current system in other organs?

Be able to relate the saturation level of hemoglobin in human blood to its location in the circulatory system. Be able to use information in hemoglobin saturation graphs to support such comparisons. What is partial pressure, and how does the partial pressure of oxygen gas change across the circulatory system? How does the exchange of carbon dioxide interact with the exchange of oxygen? Since myoglobin and fetal hemoglobin have greater affinities for oxygen than does the regular adult hemoglobin explain why these other oxygen binding proteins have not replaced adult hemoglobin.

For review see the self-quiz questions #1, 5, 7, 8 and 11 of chapter 42.

BIO 107   2010
Day 7, Lecture 17, Title: Plant Nutrition.
Text Readings: Campbell et al. (2008), Chapter 37.

<u>Objectives:</u>

What are the differences between macronutrients and micronutrients of plants? Be able to identify a few of each. You should be able to propose several different hypothetical transport mechanisms that plants could use to bring any one type of nutrient into their cells. Where in the process of uptake of items from the soil do diffusion limits matter?

Describe several factors that can alter the availability to a plant of nutrients in the soil. What might the plant attempt to do to improve its access to nutrients? In what ways might plants interact with soil microorganisms to improve the plant's nutrient status and what benefit might the soil microbes get from this association? What is the rhizosphere? Be able to describe fungal interactions with plants.

You should be able to describe the major steps in the process of root nodule formation in plants. What conditions must the plant create to promote nitrogen fixation by the bacterium? What roles do flavonoids and nod factors play in the initiation of root nodule formation?

Instead of getting their essential minerals from inorganic sources what are examples of plants that obtain their minerals from organic sources and how do they manage to do this?

For review, see self-quiz questions #3-9 of chapter 37.

BIO 107   2010
Day 8, Lecture 18, Title: Excretion.
Text Readings: Campbell et al. (2008), chapter 44.

<u>Objectives:</u>

Compare a counter-current system to a con-current system in terms of their efficiency for the removal or recovery of an item. Be able to identify examples from various species that illustrate this comparison.

In terms of the environment in which they live and the activities they carry out describe why both plants and sea birds must deal with excess salts. Be able to contrast the means by which they do so.

The functions of filtration, secretion and reabsorption are carried out in mammalian nephridia. Be able to describe each process, and how it contributes to the formation of a concentrated urine. What aspects of these functions are seen in the excretory systems of planaria, earthworms, or insects? Compare the benefits and costs of using either urea, ammonia or uric acid as a nitrogenous waste in terms of their costs to produce, their solubility, and their toxicity. Be able to compare planaria, earthworms, insects and mammalian excretory systems. Which of these animals has to recover water most efficiently, and how is this need met in terms of the excretory system they have and the type of waste they excrete? When an organism carries out excretion what is it trying to achieve? How does this differ from the process of elimination?

Know the anatomy of the mammalian kidney and nephron so that you can describe the place and function of each part in the processes it carries out (structures mentioned in figure 44.14 are relevant). Review the path of blood flow from the heart to the kidney and back. How does the blood pressure change as blood passes from the renal artery past the nephron to the renal vein? How many capillary beds does this blood pass through and where are they located? How are blood and filtrate flows orientated in the mammalian kidney to maintain the osmotic gradient between the cortex and medulla of the kidney? Be able to describe how ADH acts in the nephron to alter water recovery.

For review see self-quiz questions #1-6 for chapter 44.

BIO 107   2010
Day 8, Lecture 19, Title: Movement I.
Text Readings: Campbell et al. (2008), pgs. 112-118, 558-559, 838, 842-843, 1105-1114.

Objectives:

For a given system that results in movement be able to identify the structure acting as anchorage, and be able to identify the molecular systems by which force is generated.

Be able to describe the role of cytoskeletal structures in movement. What does a motor protein do, and what are examples of motor proteins?

Be able to contrast the means by which single-celled organisms achieve movement versus ways in which multi-celled organisms manage to move. What are three ways in which the eukaryotic flagellum differs from a bacterial flagellum? In eukaryotic flagella which motor protein moves along which static cytoskeletal element? What are the analogous molecular items seen in a sarcomere of a skeletal muscle cell?

If an earthworm wishes to extend forward what muscles must it contract? If an earthworm wishes to pull back what muscles must it contract? What is a hydrostatic skeleton?

How do plants manage to move parts of their bodies? How do they generate the forces needed to achieve this movement?

Know the characteristics of each of the three major vertebrate muscle cell types.

Be able to describe the basic structures of a skeletal muscle from the organ level down through the cellular level and on to the arrangement of the major molecules in the sarcomere. What is the sequence of events that occur as muscle myosin interacts with actin in a sarcomere during muscle contraction? Know how this interaction is regulated, including how a signal received at the cell membrane results in the contraction of a skeletal muscle cell (fig. 50.29 might be helpful here). Be sure to note the role that calcium ions play in this process and the proteins with which they interact to influence the cell's response. What could be altered to make the tension generated by a muscle greater, or make it operate faster?

For review see self-quiz question #6 of chapter 50.

BIO 107   2010
Day 8, Lecture 20, Title: Plant Dispersal.
Text Readings: Campbell et al. (2008), pgs. 804-805, 809-811.

Objectives:

Be able to describe several mechanisms by which plants manage to disperse their offspring even though established plants themselves tend not to migrate. Which mechanisms are biotic and which involve abiotic means? Consider the effect of each on the range in which a plant species would be found. What can a plant do to promote biotic means of dispersal? What modifications might be expected to help promote abiotic dispersal?

Some mechanisms of dispersal are rather harsh and relatively risky for the plant's seeds. Describe the various benefits a plant species obtains from running such risks to achieve good dispersal.

Be sure to understand what pollination is, and how it is not a means of dispersing a species.

BIO 107   2010
Day 9, Lecture 21, Title: Hormones.
Text Readings: Campbell et al. (2008), pgs. 207-210 and chap. 45.

Objectives:

Be able to describe similarities and differences between hormones and: Pheromones, secondary messengers, and items used in synaptic or paracrine signaling. Be able to describe how hormonal regulation of an organism's functions makes use of cellular signal transduction systems. Be able to contrast features of hormonal control of responses between those involving changes in gene expression to those involving changes in the activity of existing proteins, and be able to name an example of each.

What is the difference between endocrine glands and exocrine glands? Be able to name examples of each as found in a mammal (figures 45.10 and 41.14 may be helpful). What is an example of a mammalian organ that is both an exocrine and an endocrine gland?

How does the mode of action of hydrophilic hormones generally differ from that of hydrophobic hormones? What are the general molecular classes of hormones found in mammals?

Be able to describe how negative feedback loops can help regulate the secretion of hormones and maintain a desired state. How is negative feedback seen in control of the thyroid and what other glands are involved (see fig. 45.18)?   Be able to recognize how this does not involve the use of antagonistic hormones.

Be able to describe how homeostasis is achieved in the maintenance of blood calcium and blood glucose levels and the use of pairs of antagonistic hormones in these examples. Know the organs, hormones, and physiological effects involved. Consider the role of receptors, what items they must have binding sites for, and what the effect of their binding or not binding of those items should have on secretion of relevant hormones.

Be able to describe the relationship between the hypothalamus and anterior and posterior pituitary glands. In each case how do hormones get from the hypothalamus to either side of the pituitary? What is the functional role of the relationship between these endocrine glands? What is a portal system and what role does it play between these glands?

For review, see self-quiz questions #2-6 for chapter 45.

BIO 107   2010
Day 9, Lecture 22, Title: Animal Reproduction.
Text Readings: Campbell et al. (2008), chapter 46.

Objectives:

Be able to compare the costs and benefits of sexual versus asexual reproduction.

What is a cloaca and what major organ systems normally feed into it? What is an example of an animal species that has a cloaca? Know the basic structures associated with the mammalian reproductive system, both males and females.

The sequence of steps in the menstrual and ovarian cycle, and how these relate to changes in concentrations of hormones, are worth knowing (Fig. 46.14 is a good summary). What hormones are involved and what are their effects at each stage of these cycles? What roles do the follicle and *corpus luteum* play, and when do they play them? Contrast the effects of key hormones in the mammalian female with the effects of similar hormones in the mammalian male: For instance, be able to contrast the role of FSH and LH in control of male and female reproductive organs, and how their secretions are controlled. What are the differences between mammalian oogenesis and spermatogenesis?

The induction of labor is a good example of a positive feedback system. Be able to describe in what way this is so, and in what way ovulation is also an example of a positive feedback system. Be able to describe examples that illustrate negative feedback seen in both male and female mammalian reproductive systems.

Be able to describe at least two methods of contraception used by human males and two used by females. What is the mode of action of each?

For review see self-quiz questions #3, 6 and 8 at the end of the chapter.

BIO 107   2010
Day 9, Lecture 23, Title: Transport II.
Text Readings: Campbell et al. (2008), Chapter 36.

Objectives:

What is water potential, and how does water movement relate to water potential gradients? What factors influence the water potential and which of these do plants actively manipulate? If you understand the concept of water potential you should be able to describe the flow through the xylem and through the phloem, or even through the human circulatory system, in terms of the factors that contribute to water potential gradients.

Be able to describe the flow of water from the soil through the roots, up the stem, and out of the leaves of a plant. What structures and cell types are used by vascular plants to allow, or to regulate, the flow of water through their bodies?

How is root pressure generated? Does creating root pressure cost the plant metabolic energy; if so what is this energy used to do? Be able to identify the molecular events that connect metabolic energy use by the plant to a final pressure in the root. In what cells and region of the root is root pressure created? Under what conditions will root pressure be used, and what items does it typically move? What components of water potential does the plant modify to produce root pressure? Be able to describe the structure of the Casparian strip and its role in both root pressure and during transpirational flow.

Describe how the transpiration of water results in the bulk flow of water from the soil to the leaves. Does this flow cost the plant metabolic energy; if so how? What structures in the leaves of vascular plants allow the plant to control transpiration? What functions do guard cells and cuticle play in the control of transpiration, and how do they do it? How else could a plant modify its structure to alter its rate of water loss?

How is bulk water flow generated in phloem? What cells are involved in apoplastic phloem loading, and how is that loading achieved? What items are typically moved through the phloem? What areas of a plant are considered to be "sources" and what areas of a plant can be considered to be "sinks"?

For review see self-quiz questions #2-9 and 11 of this chapter.

BIO 107   2010
Day 10, Lecture 24, Title: Eukaryotic Diversity II: Chordates and Vertebrates.
Text Readings: Campbell et al. (2008), pgs. 445-446, 513, 525-528, 654-655, 661-663,
chapter 34, and Appendix E.

Objectives:

What characteristics distinguish each of the following groups? Be able to name specific examples of members of each group. Also consider how the traits found in a group influences the types of habitats in which they live or the types of lifestyles they display.

Animals
Deuterostomes
Chordata
Craniates
Vertebrates
Gnathostomes
Osteichthyans
Lobe-finned fish
Tetrapods
Amniotes
Mammals
Monotremes
Marsupials
Eutherians

Be able to describe the characteristics of the amniotic egg. How have the parts of the egg been modified amongst various amniotic species?

What are examples of traits that have appeared during the course of animal evolution that are thought to involve the duplication of genes?

Be able to describe a model for the evolution of the jaw, and what preexisting structures were modified to make it?

For review see self-quiz questions #2-6 of chapter 34.

BIO 107   2010
Day 10, Lecture 25, Title: Movement II.
Text Readings: Campbell et al., (2008), pgs. 1112-1117, fig. 40.5 (connective and muscle tissues), and the chapter on Biomechanics in the BIO 107 Lab Manual.

Objectives:

Contrast the connections and uses of tendons versus that of ligaments.

Be able to use the law of the lever in calculations to rank lever systems in terms of their relative mechanical or speed advantages. If given a joint, be able to propose a possible flexor or extensor muscle for it by indicating the possible anchorage and insertion points for each muscle relative to that joint. Consider how the muscle and its attachment points could be changed to improve either the mechanical advantage, or the speed advantage, of that joint.

Be able to describe examples of biological joints that are relatively high in mechanical advantage, or high in relative speed advantage. How are these arrangements adaptive for the animal that has it?

Be able to describe specific examples of animals that dig, run, or fly. Be able to describe adaptations that are evident in the skeletons and muscles of animals that use each one of these types of movement. If shown several skeletons be able to compare them in terms of specific adaptations present in them that would make them adaptive for digging, or running, or flight.

How are bones and joints altered to deal with compressive forces?

BIO 107   2010
Day 11, Lecture 26, Title: Study of molecular diversity.
Text Readings: Campbell et al., (2008), pgs. 97, 405 (fig. 20.9).

Objectives:

Be able to list several properties of molecules that can be used to separate them from each other. What is a separation technique that could be used to separate items according to each molecular property?

What would be differences in the approach taken when trying to isolate a hormone versus a receptor for that hormone? Be able to describe differences in the molecular abundance and properties of a hormone versus its receptor, and how this might influence the approaches and techniques of isolation that might have to be used.

Identify and describe ways to get around some of the problems involved in isolating and purifying a molecule intact out of a tissue of many cells. Why is one method of purification rarely sufficient to achieve a completely pure sample of one type of molecule?

What information about a molecule can be obtained by each of the following techniques? Centrifugation, chromatography (consider the various column and thin-layer types), and electrophoresis. Be able to describe broadly how each of these techniques works, including the molecular property that each uses to achieve molecular separation.

BIO 107   2010
Day 12, Lecture 27, Title: Action potentials.
Text Readings: Campbell et al. (2008), pgs. 1047-1056.

Objectives:

Be able to describe the sequence of events involved in the propagation of an action potential, and note which of these help define action potentials as an "all-or-none" phenomenon.

What are the different parts of a vertebrate neuron called? What type(s) of ion channels are present in the plasma membrane of a typical neuron, and where in the cell are they located?

Before an action potential what is a typical magnitude of the neuron's membrane potential? What are the relative concentrations of K^+ and Na^+ inside of, and outside of, the cell before an action potential? Be able to describe how these ions got put into in this distribution and how this relates to the resting potential the cell produces. What active transport system is needed to make these conditions be present so that an action potential can happen in a nerve cell? What passive transport systems are needed to establish the resting potential across the membrane? What passive transport systems are used during the action potential?

What is hyperpolarization, and how does it differ from depolarization? If Na^+ enters a typical nerve cell which is at rest, will the membrane potential hyperpolarize or depolarize? What if the Na^+ left the cell? What would happen if Cl^- entered or left the cell?

Why is it difficult to start a new action potential at a section of a cell's membrane that is experiencing a refractory period? Be able to describe how the firing of one action potential induces a new action potential further down the axon, but not back towards the cell body.

Be able to describe the effect of a myelin sheath on the rate of propagation of an action potential. Be able to describe saltatory conduction, and how the action potential is propagated from node to node down an axon.

What are some non-neuron cells that have action potentials in animals? What are some non-animal species that have action potentials? What features of these other action potentials would be similar to those seen in animal neurons?   What would a non-animal species be doing with their action potentials?

For review see self-quiz questions #1, 2, 3, 7 and 8 at the end of this chapter.

BIO 107   2010
Day 12, Lecture 28, Title: Synapses.
Text Readings: Campbell et al. (2008), pgs. 120-121, 1056-1061, 1078-1080.

Objectives:

What are the important structural and functional differences between chemical and electrical synapses. What structure(s) must be present between eukaryotic cells to allow for an electrical synapse to exist? What sort of structure(s) must be present, and where, for a chemical synapse to exist? Synapses are structures though which cell-to-cell communication occurs, what are some examples of cells in humans that carry out such communication across synapses?

Chemical synapses involve elements of signal reception and transduction systems. Be sure that you see this connection clearly and can identify the elements. In terms of just a single neuron's synapse, what is the signal, how is it transduced, and what might be the response of that cell?

What must happen in the presynaptic cell for it to transmit a signal to another cell? What then happens in the postsynaptic cell? The sequence of events, what structures are involved, the function of each, and how this can lead to the firing of an action potential should be clear to you (a close study of figure 48.15 might be useful here). Sending a signal across a synapse is one thing. How is the synapse reset so that the next signal can be sent?

Do all synapses result in the stimulation of an action potential in the postsynaptic cell? Do all synapses contribute equal effects on the postsynaptic cell? Can you describe how summation can work to produce complexity and information integration?

Be able to describe several ways in which the firing of a post-synaptic neuron could be modified over the long term.

Be able to propose several reasonable mechanisms for how a neuron could have its growth and formation of new synapses influenced by cells around it.

For review see self-quiz questions #4, 5, 6 at the end of chapter 48.

BIO 107   2010
Day 12, Lecture 29, Title: Nervous Systems.
Text Readings: Campbell et al. (2008), pgs. 1048, 1064-1078, 1080-1084.

Objectives:

Be able to describe the differences between a sensory neuron, an interneuron, and a motor neuron in terms of their functions and connections. The roles of these in both the human knee-jerk and *Aplysia* siphon-withdrawal reflex arcs are good examples to work through in this context. Are only neuron cells involved in information transfer, or are other cells also involved?

Contrast both the structure and the antagonistic effects of the parasympathetic and sympathetic divisions in terms of the classical "flight or fight" response. For example: Which division has ganglia closer to the spinal cord? What are effects each system has when activated?

Be able to describe the basic organization of the vertebrate nervous system down to the level of divisions, and be able to name specific areas in, and functions of the forebrain, midbrain, and hindbrain of the vertebrate brain. Describe how the nervous system functions to compare and integrate external stimuli, its role in helping to maintain internal homeostasis, as well as its role in planning. Which areas of the CNS are most ancestral and which are most derived and modified in our lineage?

Describe several methods of monitoring brain activity. Is the brain static or malleable? Be able to give examples to illustrate the reasoning behind your answer.

For review see self-quiz questions #2, 3, and 5 of chapter 49.

BIO 107   2010
Day 13, Lecture 30, Title: Senses I.
Text Readings: Campbell et al. (2008), pgs. 1087-1105.

Objectives:

For an organism to sense an environmental cue what must it have? For it to respond, what must it do? Are all environmental signals able to be sensed by all organisms? When a signal is sensed by a mammal what happens to the frequency of action potentials that are sent through sensory neurons?

Be able to describe the important structures involved in the senses of taste, hearing and sight in humans. In each case be able to describe the receptor system, and the steps in signal transduction in the sensory cells involved that occur and how they influence action potential firing in an associated sensory neuron. For instance, how the change in shape in rhodopsin in the eye results in a change in the frequency in action potentials in a neighboring bipolar cell should be clear to you. Contrast the human sense systems of taste, hearing and sight in terms of the amount of processing or modification of the signal information that occurs before information is sent to the brain. If processing is done, is it done by the sensory cells, neurons, or by other structures? If given a diagram, be able to locate such structures and locate the sensory cells and the sensory neurons involved in human taste, hearing and sight (figures 50.8, 50.18, 50.23, and in the handout are good practice here). Consider how these senses differ in terms of the nature of the signal, the relative energy content of the signal, and the speed of change in the signal that can be detected.

Be able to compare and contrast the elements of signal transduction used in taste, hearing, and sight sensory cells to elements of signal transduction used at a neural synapse.

For review see self-quiz questions #3, 4, and 5 of this chapter.

BIO 107   2010
Day 13, Lecture 31, Title: Senses II.
Text Readings: Campbell et al. (2008), pgs. 1087-1092, 1094-1096, 1099-1100.

Objectives:

How does the energy in an environmental signal have to compare to the noise in the environment in order for the signal to be detected by an organism? What is the typical type of molecule that is used by life as signal receptors and what must happen to that molecule for a signal to be received?

Do senses allow a complete, accurate, and truthful perception of the environment by a species?

How does information about the intensity, or other features, of a signal get sent through the animal nervous system when each action potential is just like any other action potential?

What is the difference between a phasic receptor and a tonic receptor? What advantage do we get from having both types of receptor systems?

Be able to describe several different molecular mechanisms that might be used to receive an environmental signal. Be able to describe common features of how various types of signals can be sensed by life forms, and describe how such sensing is adaptive for the organism that has each sense.

For review see self-quiz questions #1 and 2 of chapter 50.

BIO 107   2010
Day 13, Lecture 32, Title: Comparative Physiology.
Text Readings: Campbell et al. (2008), pgs. 708-723, 862-872, 881, 891-893, 900-903, 917-918, 920-921, 925-927, 954-960, 968-969, 1000-1001.

Objectives:

Be able to describe how the traits present in the groups of animals covered (fish, frogs birds and other reptiles, and mammals) helps makes each of the animals adaptive for the typical habitat in which each is found.

What effect can endothermy or ectothermy have on the demands for gas exchange and on the circulatory system found in a species? What are the costs and benefits of ectothermy compared to endothermy, and what types of habitats would you expect to find each? How can counter-current exchange systems be used to retain heat, and what other adaptations for heat retention are used by some vertebrates?

What adaptations are seen in some vertebrate groups that makes them more adaptive to different feeding patterns, such as carnivory versus herbivory?

In what ways are the circulatory systems found in frogs and in some reptiles adaptive for doing gas exchange through various systems in their bodies? Fish have relatively simple circulatory systems, how is this possible?

Be able to describe several adaptations found in land dwellers, and others found in marine species, that address the need to retain water.

What are the costs and benefits of external compared to internal fertilization? What adaptations tend to be associated with each?

Not just the adults, but also earlier stages in the life cycle must be adaptive. Describe traits found in each of the following and how they make them more adaptive: tadpoles, chicks in the egg, and mammalian fetuses.

BIO 107   2010
Day 14, Lecture 33, Title: Animal Behavior.
Text Readings: Campbell et al. (2008), Chapter 51.

Objectives:

How does a behavioral response compare with a growth response? What is similar and what is different? We call behavior an emergent property, but from what does it emerge? What components are needed in animals in order to have a behavior?

What must be seen in a behavior for it to be taken as evidence of habituation? What is needed to show a taxis? A kinesis? A fixed action pattern? Be able to describe these examples in terms of likely signal reception, transduction, and response processes at the cellular, tissue, and organismal levels.

How would you determine whether a behavior is innate or is learnt? Be able to describe examples of each type of behavior. How does operant conditioning differ from classical conditioning? Be able to describe examples of each that might be observed in the wild. What is imprinting and is it purely innate or learnt behavior? Is the capacity for, or use of, language an innate or a learnt behavior?

Is altruistic behavior adaptive for an individual or for a species or for both or neither? How so? Relate this to specific examples of altruistic behaviors. Be able to calculate relative genetic relatedness and describe the arguments that relate this to how altruistic behaviors may be adaptive.

For review, see self-quiz questions #1, 3, and 6 at the end of this chapter.

BIO 107   2010
Day 14, Lecture 34, Title: Non-Animal Behaviors.
Text Readings: Campbell et al., (2008), pgs. 207, 777-778, 821-824, 838, 842-843.

Objectives:

Be able to contrast the text's definition of behavior with the definition presented in this lecture. How do the systems described for *Myxococcus xanthus*, protists, and for plants fit the definition of behavior given in this lecture?

Be able to contrast a behavioral response with a growth response. Which type of response might each of the following be: The formation of an anthrax endospore? The swimming of a euglena towards the light? The sleep movement of a bean plant? The courtship of stickleback fish? In each case consider what specific information you would need to have to confirm your answer.

Consider what is needed in order for a behavior to be displayed by an organism. What molecular and cellular mechanisms must be present? Consider if these mechanisms are only found in animals, and only in the nervous and muscle tissues of animals.

BIO 107   2010
Day 1, Lecture 1, Scientific Method.
             (aim for 40 min, after introduce course...)
Text Readings:
Course Information and Policies Handout, text pgs. 18-24, and chapter 1 of the Lab Manual.

Topics to cover:
        Course Business
        Scientific Method
        Example: Kettlewell's study

Course Business
        Handouts
        Fill out 3X5 card, name, contact info, goals in life, etc...
        Office hours   M-F, 12:30-1:30, Wed 10:00-1:30 in 1122 Comstock
        Describe: Calendar, grading scheme, objectives, lecture notes, web site, sample exams
                     syllabus, attendance, check e-mails... etc.
                Success and failure patterns in course seen in the past.
        Wed. last day to drop with full tuition refund.
        Get copy of lab manual.
        Lab meeting today (field trip)

Scientific Method

        Compare this process to value/ethical decisions
        Limits of scientific method
                        What is right? Beautiful? Moral? Good?
                Allows formation of better models of the universe, does a poor job with ethics..
                        Ex: Know how to make a nuclear bomb, does
                                not tell us whether or not to use it.
                Science is limited to model production.
                        A good model describes known situations, clearly defines its limits, and
                                makes testable predictions.

Scientific method (Campbell and Reece, 2005: fig. 1.25)
        Review steps in the process
                Explanatory system, research model, hypothesis generation, tests, data analysis
                        conclusions, and modification of the explanatory system.
                Produces a testable model. Model accounts for certain past observations and
                        results, but also makes testable predictions. Models are not static but are
                        modified as new information is incorporated. Thus a dynamic process
                        (relate it to what will happen in course in lab and lecture?)
                        (Fig. 2.1, BIO 103 lab manual, Glase 2002)

Explanatory system, previously known information
Inductive reasoning, leads to a generalization from known data
    This can lead to a research model (often called a research hypothesis).
        A research approach (but typically requires little testing)
        So does not require checking outside of the known data.
        While limited, this may produce an initial model of known data?
Deductive reasoning, leads from a generalization, but put in a "If... then..." format that
        implies reference to new data yet to be collected.
    From this research hypothesis can then get to specific testable predictions.
    These predictions are called hypotheses; there are better and worse ones...
        Non-specific hypotheses, often interesting issues but hard to evaluate
            Ex: Life is only on Earth?
                Life exists elsewhere in the universe?
            Ex: All leaves are green.
                Not all leaves are green.
        Specific hypotheses, very narrow, but give specific expected results
            Generally set up as a pair of hypotheses, for clarity
        Use of null and alternative hypotheses... Two general types:
            1.) Dealing with differences between two sets of data
            Null hypothesis Ho, no difference between two sets of data
            Alternative hypothesis Ha, a difference exists
                Note role of chance
                Ex: This coin is honest $(H = T)$. (very specific)
                      This coin is not honest $(H \neq T)$ (rather vague)
            2.) Dealing with correlations between two different items
            Null hypothesis, no correlation exists (very specific)
            Alternative hypothesis, there is a correlation (rather vague)
Test of hypothesis
    observational studies
        Some items can not be manipulated by us, but are still important
        Ex: Does the frequency of the allele associated with sickle cell anemia
            differ in people who have lived for generations in the presence of
            malarial parasite? We do not add or remove this parasite, nor do
            we manipulate the allele. So this is an observational study.
    In either case collect data
    In experimental studies produce a control situation for comparison
        a situation in which one factor that is present in the treatment group is not
            present
        This is an attempt to control for variation.
            Experimental design is often about eliminating extraneous
                variation.

Compare observed and expected results
    Note how Ho gives very specific expectations,
        while Ha's expectations are vague
    So typically use Ho's expectations as basis for evaluation.
Carry out data analysis → use of statistics
        Could the results be due to chance?
        If chance can produce the observed differences, then a difference
            is not a "proof" of difference.

Standards of proof
    (see overhead, file Larson_proof.jpg)
    Typically use statistical analyses to determine if two sets of data differ
    Deals in probabilities
        differences can occur by chance
        if difference occurs by chance 5% of time or less,
            then we assume the differences may be real
    Note, this is different from proof
        To "prove" a model have to test every possible case; rather impossible
        Instead it is more rigorous to disprove something
            since it takes just once case to refute a model
            typically the null hypothesis gives the most specific expectations
                and so refuting the null hypothesis is easiest
        We seldom "prove" things in science. But we often disprove things.

Form conclusion
    Can either disprove the model, and so need to refine or limit it to certain
        conditions? I.e. true for mammals but not for bacteria?
    Alter explanatory system.... add the information to the published body of
        information.

Repeat aspects of the above process many times.
    Eventually get enough information for a publication.

Ex: Kettlewell's studies of moth
        Peppered moth (*Biston betularia*), in England
        Already known (explanatory system):
            (Figure Gould and Keeton, 1996, fig. 17.31)
            moths active at night, rest during day
            Often rests on the bark of trees.
            Soot from coal fires altered color of bark.
            Dark form first reported in 1848, typical is light form. By 1898, 98%
                were dark colored.
            This was associated with darker colored moths
                    "industrial melanism".

1926, Harrison, proposed that some agent in soot was ingested by moths, this altered their color, and then this trait was inherited. (sort of Lamarkian?)
    Tested by breeding in absence of soot.
    So dark trait is heritable. I.e. coloration is genetically determined.
     (Added this to explanatory system)
   1950s, Kettlewell's studies, mark and release
  His research model was that the differences in the moth colorations was associated with differences in the degree of predation in different locations.

  From that model he used deductive reasoning: If.... then....

   "If the increase in the dark moth is due to decreased predation because of their increased crypticity on the soot-darkened bark surfaces, and the decrease in the light morph is due to increased predation because of their reduced crypticity on the soot-darkened bark surfaces then (1) we should be able to collect significantly more light moths than dark moths from soot-free woodlands in unpolluted areas; and (2) we should be able to collect significantly more dark moths than light moths from soot-darkened woodlands near industrial areas."
(Glase, 2002; pg 27)

  State Ho and Ha, in this first case doing two experiments. Initially an observational experiment. Went to two areas of woods and observed the colors of catachable moths in each.
    Ho: In the area used, the number of dark colored moths will equal the number of light colored moths (i.e. 50% and 50%).
    Ha: In the area used, the number of dark colored moths is different from the number of light colored moths.
(Glase 2002; table 2.1, pg 28:)
  "Numbers and percentages of light morphs and dark morphs of peppered moth (*Biston betularia*) collected in soot-free and soot-darkened woodland areas." (Kettlewell, 1959)

	Number (%) of Moths Collected	
Morph Type	Soot-Free Woodland	Soot-Darkened Woodland
Light	324 (99.1)	144 (9.3)
Dark	3 (0.9)	1403 (90.7)

  Total =   327   1547

  Could these values have been produced by chance?
  Do these data "prove" the hypothesis? Need to do statistical analysis...
  Are there other reasonable hypotheses? Must these results be due to differences in predation? I.e. what if dark moths leave areas lacking soot? What if they differ in other ways? What if one happened to lack light colored moths just by chance?

4

Next Kettlewell did a release and recapture experiments. An experimental test, as manipulating the area by introducing test subjects.

(from table 2.2, pg. 29, Glase 2002:)
        "Number of light morphs and dark morphs of peppered moth (*Biston betularia*) released and recaptured in soot-free and soot-darkened woodland areas." (Kettlewell, 1959)

Morph Type	Soot-Free Woodland		Soot-Darkened Woodland	
	Number Released	Number (%) Recaptured	Number Released	Number (%) Recaptured
Light	393	54 (13.7)	137	18 (13.1)
Dark	406	19 (4.7)	447	123 (27.5)
Total =	799		584	

Again, note that this is really two experiments.
What is Ha and what is Ho in this case?
        Ha; Percentage of released moth types that are recaptured should differ after they are allowed to live in the area.
        Ho; Percentage of released moth types that are recaptured should be similar after they are allowed to live in the area.
These results are consistent with his model, but notice that it is still not proof. There are many other possible explanations for these data.

Next Kettlewell checked actual predation.
        Would differential predation account for the differences seen?
(from table 2.3, pg. 29, Glase 2002:)
        "Number and percent (%) of light and dark morphs of peppered moth (*Biston betularia*) eaten by birds in soot-free and soot-darkened woodland areas." (Kettlewell, 1959)

Morph Type	Number (%) Eaten by Birds	
	Soot-Free Woodland	Soot-Darkened Woodland
Light	26 (13.7)	43 (74.1)
Dark	164 (86.3)	15 (25.9)
Total =	190	58

Is this consistent with Kettlewell's hypothesis?

Is it proof? What additional information does this last experiment present that the other two experiments do not relative to Kettlewell's initial research model? Are all three of these experiments equivalent in terms of relevance to the original research model?

Note: Have not covered statistical testing, will get this in lab.

Conclusion: Scientific method requires testing and reevaluation models.

This is useful as models often have testable aspects, and can be refined.

But the models are incomplete and imperfect..

Do not believe claims, must have tests.

Doubt everything I tell you in this course, the models you are given are likely to be incomplete...

Objectives:

Know the stages of the scientific method. What should a good scientific model be able to do? Be able to contrast inductive and deductive reasoning. How do observational studies differ from experimental studies? Which is more likely to have a control group, and what is the purpose of a control group? Just because a model has been produced and has evidence to support it does that make it valid in all cases? Does Kettlewell's model of the affects of predation on moths apply to all species of moths, or does it have specific limits?

Be able to describe and contrast the uses of null and alternative hypotheses. Explain the purpose of these statements in the scientific method: Which gives the most specific prediction? Given an example of an experiment be able to state null and alternative hypothesis that are appropriate for that specific experiment. Understand the difficulties of "proving" a hypothesis compared to the relative ease of disproving a hypothesis. Can you relate this to why we use the null and alternative hypotheses as a means to clarify our thinking?

Given an example of part of a scientific investigation be able to describe the aspects of the scientific method it contains. For instance, given the description in the lab manual you should be able to describe how Kettlewell used various stages of the scientific method at various points in his studies.

Needed overheads and items:

Fig. 1.25, Scientific method (Campbell and Reece, 2005)
Standards of proof: file larson_proof.jpg

Fig. 2.1, Scientific method, pg. 31, 103 lab manual (Glase, 2002).
        (see sci_meth.jpg)

Images from Gould and Keeton (1996, fig. 17.31)
        whitemoth2.jpg, whitemoth3.jpg

Fig. 1.19, (Campbell and Reece, 2002) Scientific method

Make tables with Kettlewell data for three expts.

Bring in a non-green leaf?

References:

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Gould J.L, W.T. Keeton- 1996-<u>Biological Science</u>, 6th edition. fig. 17.31, pgs. 474-477. Norton & Company, N.Y.

Larson, G- 1994-<u>The Curse of Madame "C"</u>- pg. 35. Universal Press Syndicate. Kansis City, Missouri

Kettlewell H.B.D- 1959-Darwin's missing evidence- Scientific American 200: (#3) 48-53

Related Items: (Not required but of possible interest..)

In these publications the application of the scientific method allowed the study of something previously thought to be beyond the reach of science or to do so in new ways: The first two deal with "out-of-body" experiences, and the third involves the use of robots (!) to study insect behavior:

> Ehrsson H.H- 2007-The experimental induction of out-of-body experiences- Science 317: (#5841, 8/24) 1048
>
> Lenggenhager B., T. Tadi, T. Metzinger, O. Blanke- 2007-Video *ergo sum*: Manipulating bodily self-consciousness- Science 317: (#5841, 8/24) 1096-1099
>
> Halloy J., G. Sempo, G. Caprari, C. Rivault, M. Asudpour, F. Tâche, I. Saïd, V. Durier, S. Canonge, J.M. Amé, C. Detrain, N. Correll, A. Martinoli, F. Mandada, R. Siegwart, J.L. Deneubourg- 2007-Social integration of robots into groups of cockroaches to control self-organized choices- Science 318: (#5853, 11/16) 1155-1158

Scientific models are not perfect, and neither are experimental designs. But science is a self-correcting system. The first article notes that 19 out of a set of 77 examined articles have questionable conclusions based on flawed experimental designs. The second describes debates over observations concerning whether a species of bird is in fact still alive or is indeed extinct. The third notes a case of retraction of published studies that were found to be flawed. So even published findings are not always able to "prove" things:

> Couzin J- 2007-Epidemiologist sees flaws in papers on genes and gender- Science 317: (#5841, 8/24) 1020-1021
>
> Stokstad E- 2007-Gambling on a ghost bird- Science 317: (#5380, 8/17) 888-892
>
> Holden C- 2007-Former Hwang colleague faked monkey data, U.S. says- Science 315: (1/19) 317

The following describe some interesting views on how the scientific process actually gets done in real life. In most cases the view presented relates to a larger context of society and ethics:

> Caplan A-1995-Moral matters: Ethical issues in medicine and the life sciences- 198 pages. John Wiley & Sons, Inc. New York, New York.
>
> Koshland D.E.jr- 2007-The Cha-Cha-Cha theory of scientific discovery- Science 317: (#5839, 8/10) 761-762
>
> Kuhn T.S- 1970-The structure of Scientific Revolutions. Second edition. 210 pages. University of Chicago Press. Chicago, IL.
>
> Macrina F.L- 1995-Scientific integrity: An introductory text with cases. 283 pages. ASM Press. Washington D.C.
>
> Nabel G.J- 2009-The coordinates of truth- Science 326: (#5949, 10/2) 53-54

BIO 107   2010
Day 1, Lecture 2, Title: Life and Evolution

Text Readings: Campbell et al. (2008) pgs. 1-17.

Topics to cover:
    What is Life?
        Structural/Functional features
        Definitions of life
    What is Darwinian Evolution?
        Preconditions and assumptions

What is Life?
    Structural/Functional features
    In Discussion format: Ask, have students write out ideas...:

What is a definition of life (on Earth)?
        Ask for structural/substance features.
        Ask for functional features.
Some structural features:
        Carbon-based compounds: carbohydrates, lipids, proteins,
                    nucleic acids (fig. 1.10)
        Membranes
        Nucleic acid genetic material
        Cellular   (fig. 1.8) (fig. 1.4; Campbell and Reece, 2002)
        Water based
Some functional features:
        (fig. 1.3 processes in life)
        Reproduction (of organism): Soap bubbles do this...
        Replication (of molecules): DNA does this...
            Notice how this differs from reproduction.
        Order (entropy generating outside of selves)
        Growth and development
            Stereotypical patterns of development
        Energy utilization and transformation (fig. 1.5)
        Interaction with the environment (protein sensors, import, export across
            membrane)
        Homeostasis (inside distinct from outside)
                implies metabolic regulation
        Maintain self-information (with variation, only
            seen in populations, implies evolution)
            inheritance
        And life undergoes Evolution?

Definitions of life:

      Here are some sample definitions of life:

"I define life... as a whole that is pre-supposed by all its parts."
                - S.T. Coleridge (ca. 1820; from Cairns-Smith, 1982)-

"We shall regard as alive any population of entities which has the properties of multiplication, heredity and variation."
                - J. Maynard Smith (1975; from Cairns-Smith, 1982) -

"Life is the system of nucleic acid and protein polymerases with a constant supply of monomers, energy and protection."
                - Kunin (2000) -

"A system which is self-sustaining by utilizing external energy/nutrients owing to its internal process of component production and coupled to the medium via adaptive changes which persist during the time history of the system."
                - Luisi (1998) -

"It is the continuity of cellular operation that defines the phenomenon of life that distinguishes what we call "life" from everything else we see around us."
                - Fleishaker (1994) -

"... it might be claimed that the most important fact about them [living things] is that they take part in the long term processes of evolution."
                - C.H. Waddington (1968; from Cairns-Smith, 1982) -

The official NASA definiton of life:

"Life is a self-sustained chemical system capable of undergoing Darwinian evolution."
                (from: Luisi, 1998)

      So life is what evolves? What then is evolution?...

What is Darwinian evolution?
So life is capable of evolution, what then is evolution?
      fig. 1.14, classification of life
In Discussion mode still: Ask students to write out ideas:
            What preconditions are needed for it to occur?

1) Excess population production.
      More born than can live to reproduce.
2) Variation amongst individuals of a species (need populations).
3) Some characteristics give survival/reproductive benefit.
      i.e. Natural selection occurs. (Fig. 1.20)
4) The above characteristics are heritable.
5) Vast amounts of time. Over which reproductive cycles occur.

Also often overlooked: Life already exists.

A nice set of circular arguments. Life is what evolves, and evolution is done by life...

Discuss:

Do individuals evolve? Not in biological evolutionary sense...

Does Darwinian evolution account for the origin of life?
      A good model has limits...

Biological evolution vs. common term usage.
      "Evolution" often used to mean just "change". That is not the biological usage.
      Biological evolution refers to formation of new species.
      Watch out for semantic confusion.

Other aspects of evolution added since Darwin's time:
          mechanism of the inheritance of traits
          not all traits are adaptive, neutral traits
             de-throning of natural selection as only means
          the role of chance events
          Much longer periods of time now recognized.

Objectives:

Be able to describe several structural features of life, and several functional features of life, as found on Earth. Describe how reproduction differs from replication. If given a draft definition of life be able to critique it in terms of what important features of life it includes and propose several features that it does not cover.

Be able to identify five essential preconditions for Darwinian evolution to occur. Characterize biological evolution in terms of: The time scale over which it occurs. The ability of individual organisms to undergo it. A likely mechanism that drives it.

Contrast the meaning of the term "evolution" as used in the context of the biological theory of evolution versus its meaning in common speech.

Needed overheads and items:

Fig. 1.8, eukaryotic and prokaryotic cells
Fig. 1.4, eukaryotic and prokaryotic cells (Campbell and Reece, 2002)
Fig. 1.10, Model of DNA structure
Fig. 1.3, processes carried out by life
Fig. 1.5, energy and nutrient cycling in ecosystem
Fig. 1.14, classification of levels of life
Fig. 1.20, natural selection

References:

Cairns-Smith A.G- 1982-Questions of evolution. Chapter 3, Pgs.78-118, in <u>Genetic takeover and the mineral origins of life</u>. Cambridge University Press, Cambridge.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-<u>Biology</u>. Eighth edition. Pgs. 1-17. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figure 1.4. Benjamin Cummings Press. San Francisco, CA.

Fleishaker G.R- 1994-A few precautionary words concerning terminology- in, <u>Self-production of Supra molecular structures: From Synthetic Structures to Models of Minimal Living Systems</u>- G.R. Fleischaker, S. Colona, P.L. Luisi editors. Kluwer Academic Publishers, Dordrecht. NATO ASI Series C, vol. 446, Pgs. 33-41

Kunin V- 2000-A system of two polymerases - a model for the origin of life- Origins of life and evolution of the biosphere 30: 459-466

Luisi P.L- 1998-About various definitions of life- Origins of life and evolution of the biosphere- 28: 613-622

Related Items: (Not required but of possible interest..)

For some additional, and interesting, views on the definition of life see:

Cleland C.E., C.F. Chyba- 2002-Defining "life"- Origins of life and evolution of the biosphere- 32: 387-393

Ruiz-Mirazo K., J. Peretó, A. Moreno- 2004-A universal definition of life: Autonomy and open-ended evolution- Origins of life and evolution of the biosphere 34: 323-346

Sagan C- 1974-On the terms "biogenesis" and "abiogenesis"- Origins of life 5: 529

Varela F.J- 1994-On defining life- in Self-Production of Supra molecular Structures: From synthetic structures to models of minimal living systems- G.R. Fleischaker, S. Colona, P.L. Luisi editors. Kluwer Academic Publishers, Dordrecht. NATO ASI Series. Pgs. 23-31

Some nice overviews of the origin and history of life on earth are given by:

Fry I- 2000- The Emergence of Life on Earth: A Historical and Scientific overview. 283 pgs. Rutgers U. Press, New Brunswick, N.J.

Kamminga H- 1988-Historical perspective: The problem of the origin of life in the context of developments in biology- Origins of life and evolution of the biosphere- 18: 1-11

This text describes the history of spontaneous generation and evolution in the 1800s:

Strick J.E. 2000- Sparks of Life. Darwinism and the Victorian debates over spontaneous generation. 201 pages. Harvard University Press, Cambridge, MA.

Life may take on new meanings as we alter it. The formation of artificial life forms produced by us via biotechnology is considered in the following articles:

Gibbs W.W- 2004-Synthetic life- Scientific American 290: (#5) 74-81

Kaiser J- 2007-Attempt to patent artificial organism draws a protest- Science 316: (6/15) 1557

Here is an interesting article that describes how life can use interactions to produce many new outcomes.

Tsai T.Y-C., Y.S. Choi, W. Ma, J.R. Pomerening, C. Tang, J.E. Ferrell jr- 2008-Robust, tunable biological oscillations form interlinked positive and negative feedback loops- Science 321: (#5885, 7/4) 126-129

BIO 107   2010
Day 1, Lecture #3, Title: Carbon chemistry

Text Readings: Campbell et al. 2008; Chapters 2 and 4; skim Chapter 3.

Topics to cover:
    Emergent Properties
    Atoms and bond numbers
    Electronegativity
    Bond Types
    Molecular shapes and polarity
    Isomers and carbon asymmetry
    Redox States
    Functional Groups

    Emergent properties
        With rise in organization new properties emerge
            which could not have been predicted.
        Ex: How does cell theory anticipate brains? Doesn't?
            How does atomic composition of humans
                predict organ systems? (see table 2.1)   Doesn't?
        Wholistic vs. reductionistic approaches...
            Benefits and pitfalls of each....
        Here will see that SOME aspects of organic molecules can be predicted
            from the elements and bond types found in them...

    Atoms and possible molecular shapes, consider if use just one element
        Relate electron valences to number of bonds   (fig. 4.4)
        H   Only one bond, only linear and only two atoms
        O   Two bonds, but too reactive, we'll see why
                Forms O_2, so locks up.
        N   Three bonds, but can make N_2 and lock up.
        C   Four bonds, makes many shapes possible
        can not lock up C to C. So can make very complex molecules
        When combine atom types get even more complex organic molecules.

    Atoms and **electronegativity**
            Related to affinity of nucleus for electron.
            Fig. 2.8, energy level electron falls to related to stability.
            Fig. 2.9, Periodic table of elements
            Relate electronegativity to: completion of octet, rises as shell fills
                atomic size,   drops with size
            See relative values given in handout (see Table 3.5, Mortimer 1971)

Bond Types

Several Bond types between atoms

All based on electrical interactions...

Rough strengths of interactions:

Covalent	20-100 Kcal/mole
Ionic	100 Kcal/mole (dry) to 0 Kcal/mole (wet)
Hydrogen bonds	0.5 Kcal/mole
Van der Waals	0.05 Kcal/mole

Covalent   atom to atom, which are not ionized (See Cram etal., 1978)

More equal electron affinity

Two types: polar and non-polar covalent bonds...

Non-polar covalent   Equal electronegativity, or small differences

H-H   C-C   C-H and S-H   (Table 3.5, Mortimer, 1971)

Polar covalent   Not enough to ionize, but enough

for unequal distribution of the electrons

C-O C-N O-H N-H

Ionic   ion to ion

for atoms at edges of periodic table, close to full outer e^{-1} shells

one favors losing, other donating   (Fig. 2.14)

This might imply that no large structure is possible.

But that would be wrong. (Fig. 2.15) Consider rocks...

Other bond types tend to be be weaker...

H-bonds   Fig. 2.16 A bond between molecules (or different regions of a large molecule)

This is what we use in our muscles to pull!

Fig. 3.2,   Also produces water tension

Note, this is a much weaker bond, NOT a covalent bond with H.

Van der Waals interactions, between two non-polar regions

See hydrocarbon side chains (fig. 4.5, hydrocarbons)

very weak, but enough to hold up geckos! (Autumn et al. 2000)

(fig. 2.UN42 Geckos)

Note that weaker interactions can be good as easily altered...

critical for enzymes to function... Weak can be good.

Molecular shape, molecular polarity, and weak bonds between molecules

Water   Fig. 2.13

Has both polar bonds and a polar molecular shape. Note how these differ.

Therefore can H-bond well, between water or with other polar bonds.

Contrast with CO_2, polar bonds and non-polar molecule.

Or two non-polar molecules that can react and produce a polar one. (fig. 2.UN44)

Molecular isomers and carbon asymmetry
> (Fig. 4.7)
> structural isomers, geometric isomers
> enantiomers, handedness
> How to tell an asymmetric carbon...
>> All four bonds to different things, not just elements but groups...
>> note in this fig. 4.8c, the -CH_3 group differs from the -COOH group.
>> so even though involve C-C bonds the central carbon is asymmetric
> How many asymmetric carbons in chitin? (fig. 5.10, chitin)
> (Students might note the carbons shown in question 4, chapter 4, pg 67 of text)
> Some drugs occur with various handedness, only one is active form (fig. 4.8)

So can tell alot if really know the properties of atoms and how they interact...

Reduction states of carbon

At least two ways to view redox state..

If ionization occurs: (as in fig. 2.14)
> Reduced: gains electrons, this only for ionizations
> Oxidized: loses electrons, this only for ionizations

Redox changes with no ionization: (more typical for carbon chemistry...)
> Reduced carbon: Has many bonds with H, few with O
> Oxidized carbon: Has many bonds with O, few with H

Consider this for just carbons (from Weber 2000, see handout; carbon_redox.jpg)
> What is most reduced form of a single carbon? (CH_4)
> What is most oxidized form of a single carbon? (CO_2)
> Intermediates? (CH_3OH, CH_2O, HCOOH)
>> Note increase in number of bonds of C with O... This is oxidation of carbon.
> What if have one C-C bond?
>> Two C-C bonds?
>> Three? Four?
> Complete pyramid. (see handout) Note tradeoffs in oxidation states.
> Point out hydrocarbons, alcohols, carbonyls, carboxyls.
> Point out redox state, relate to energy changes used by cells....

For an example of a redox reaction that involves ionization
> see fig. 2.14, for NaCl

For an example of changes in redox state of carbons in a molecule as converted to another
> Consider changes in a sugar (fig. 4.UN67a, sugars)
> Top carbon is being oxidized, middle one is being reduced
>> overall for the molecule, no change in redox state...

Functional groups

Review fig. 4.10 (Campbell and Reece, 2005)

Include

methyl, $-CH_3$,   non polar covalent, decreases water soluility
good for van der Waals interactions, see this in lipids...

hydroxyl, $-OH$,   polar covalent, increases water solubility
good for H-bonds, does not ionize normally

carbonyl, $HC=O$, and $C=O$,   polar covalent,
mproves water solubility, can H-bond

carboxyl, $COOH$ polar, can ionize, an acid
may form H-bond, negative charge may form ionic bond...
Note: It does NOT contain a hydroxyl...
the "-OH" in it should not be considered to be a
hydroxyl group.

amino, $-NH_2$,   polar covalent, can ionize, water soluble
may form H-bond or ionic bonds

sulfhydryl, $-SH$,   borderline polar covalent, water neutral
two of them can form disulfide bond, $-S-S-$
a change in the redox state of the sulfurs

phosphate, PO_4,   polar, ionizes, improves water solubility
may form ionic bonds
Note: Also does NOT contain any hydroxyls...

So if know properties of H, C, N, O, P, and S can predict a good many things about the organic molecules that contain them...

Thus these predictable properties are NOT emergent, since we can anticipate them. However we could not predict many aspects of life from these atomic/molecular properties, and those would be emergent properties...

Objectives:

Be able to relate atomic properties, especially in terms of the number of bonds and polarity of bonds that can be made, to the properties of molecules made up of those atoms. Be able to compare covalent bonds, ionic bonds, H-bonds, and Van der Waals interactions in terms of their relative strengths and properties.

Be able to order carbon groups in terms of their reduction states. What are some ways in which carbon is an ideal atomic base for organic matter? Consider the influence of electronegativity, valence, and the asymmetric carbons on the variation of the properties of molecules that can be produced.

Be able to describe and recognize different bond types that exist in organic molecules and between molecules. If given any two molecules (for instance pick any two shown in figure 5.17 on pg. 79 of our text) be able to identify all the ways in which they might interact. Or if given any one of these molecules you should be able to identify polar and non-polar bonds, functional groups, acids, bases, and asymmetric carbons in it. By examining a molecule be able to determine if it is very water soluble or not.

(We do not have time to go into the material in chapter three of the text. But you might wish to skim over the material and be sure you have a general understanding of the following items covered in it as useful background on water: Cohesion, role of water as a solvent, its ability to H-bond, and pH.)

Suggested review questions. Chapter 4: # 3, 4, and 5.

Needed overheads and items:

Text set
        Table 2.1, Human elemental composition.
        Fig. 4.4, Valences of HCNO atoms.
        Fig. 2.8, Electron energy levels.
        Fig. 2.9, Periodic table of the elements.
        electronegativity.jpg, Mortimer, 1971 (table 3.5, pg. 82)
        Fig. 2.14, Ionic bond.
        Fig. 2.15, Crystals and ionic bonds.
        Fig. 2.16, H-bond and molecular polarity.
        Fig. 3.2, Water H-bonds
        Fig. 4.5, hydrocarbons
        Fig. 2.UN42, Gecko
        Fig. 2.13, Water polarity.
        Fig. 2.UN44, Water formation.
        Fig. 4.7, Isomers.
        Fig. 5.10, chitin
        Fig. 4.8, Examples of enantiomers.
        carbon_redox.jpg (Weber, 2000)
        Fig. 2.14, ionic transfer of electrons
        Fig. 4.UN67a, sugars
        Fig. 4.10, Functional groups (Campbell and Reece, 2005)

Slidewrite files:
        c:\sww2\introbio\carbon.tc, carbon1.tc, carbon2.tc, carbon3.tc

Want handouts for students:
        Electronegativity of elements,
        Bond energies
        redox table from Weber....

References:

Autumn K., Y.A. Liang, S.T. Hsieh, W. Zesch, W.P. Chan, T.W. Kenny, R. Fearing, R.J. Full- 2000-Adhesive force of a single gecko foot-hair- Nature 405: (#6787, June 8) 681-685

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-<u>Biology</u>. Eighth edition. Chapters 2, 3, 4. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition, Fig. 4.10. Benjamin Cummings Press. San Francisco, CA.

Cram J.M., D.J. Cram- 1978-<u>The essence of organic chemistry</u>. Pg. 35. Addison-Wesley Pub., Reading, MA

Mortimer C.E- 1971-<u>Chemistry: A conceptual approach</u>. 3rd edition. pg. 82, Table 3.5. D. Van Nostrand Co., N.Y., N.Y.

Weber A.L- 2000-Sugars as the optimal biosynthetic carbon substrate of aqueous life throughout the universe- Origins of Life and Evolution of the Biosphere 30: 33-43

Related Items:

Knowledge of bonds and their interactions allows the design of robots who can climb walls, as well as self-cleaning surfaces. For instance the lotus is renowned for its "purity" and this is at least in part due to its **hydrophobic** surfaces.

Barnes W.J.P- 2007-Biomimetic solutions to sticky problems- Science 318: (#5848, 10/12) 203-204

Barthlott W., C. Neinhuis- 1997-Purity of the sacred lotus, or escape from contamination in biological surfaces- Planta 202: 1-8

Granick S., S.C. Bae- 2008-A curious antipathy for water- Science 322: (#5907, 12/5) 1477-1478

These studies that examine the strength of **H-bonds** between molecules:

Roscioli J.R., L.R. McCunn, M.A. Johnson- 2007-Quantum structure of the intermolecular proton bond- Science 316: (4/13) 249-254

Kearley G.J., F. Fillaux, M.H. Baron, S. Bennington, J. Tomkinson- 1994-A new look at proton transfer dynamics along the hydrogen bonds in amides and peptides- Science 264: (#5163) 1285-1289

From quantum theory the properties of **water** can now be accounted for, but larger molecules are still too complex to be fully accounted for just from our understanding of quantum theory.

Stone A.J- 2007-Water from first principles- Science 315: (3/2) 1228-1229

These articles look at how the interaction of **ions** with **water** can alter the **H-bond** network of water.

Ji M., M. Odelius, K.J. Gaffney- 2010-Large angular jump mechanism observed for hydrogen bond exchange in aqueous perchlorate solution- Science 328: (#5981, 5/21) 1003-1005

Tielrooij K.J., N. Garcia-Araez, M. Bonn, H.J. Bakker- 2010-Cooperativity in ion hydration- Science 328: (#5981, 5/21) 1006-1009

For a nice review of the forces that are found in the different **bond types** see:

Cotterill R.M.J- 2002-Energies, forces and bonds- Chap. 3, pgs. 23-42, in Biophysics: An introduction. John Wiley & Sons, Ltd. West Sussex, England.

For a review of **hydrophobic** interactions in lipids, including van der Waals, see:

Nagle J.F- 1976-Theory of lipid monolayer and bilayer phase transitions: Effect of head group interactions- Journal of Membrane Biology 27: 233-250

It turns out that **ionic bonds** in metal complexes are part of the way that mussels anchor themselves so strongly to underlying rocks.

Harrington M.J., A. Mask, N. Holten-Andersen, J.H. Waite, P. Fratzl- 2010-Iron-clad fibers: A metal-based biological strategy for hard flexible coatings- Science 328: (#5975, 4/9) 216-220

Messersmith P.B- 2010-Holding on by a hard-shell thread- Science 328: (#5975, 4/9) 180-181

This is a nice article that reviews **emergent properties** of life.

Balazs A.C., I.R. Epstein- 2009-Emergent or just complex?- Science 325: (#5948, 9/25) 1652-1634

BIO 107   2010
Day 2, Lecture 4, Title: Classes of Organic Matter

Text Readings:
Campbell et al. (2008) Chap. 5.

Topics to cover:

Overview
Condensation Reactions
Carbohydrates
Lipids
Nucleic Acids
Proteins

Overview
Four general groups of organic matter are?
        carbohydrates, proteins, lipids, and nucleic acids
Other groups exist... so these four are just the "biggies"...
Will cover general properties, characteristic bonds, structural features...

Condensation Reactions
Fig. 5.2, condensation reaction
Condensation Rx vs. hydrolysis Rx. Note role of water in all of this
        Note that condensation reactions are synthetic,
        and hydrolytic reactions are digestive...
condens.tif (slidewrite file), review groups that can do condensation Rxs
        alcohol and alcohol: glycosidic bond
        acid and alcohol: ester bond
        amine and acid: peptide bond
        phosphate and alcohol: phosphoester bond

Monomers to polymers, except in the case of lipids...

Carbohydrates:
        A hydrated carbon (CH_2O)
        Name often ends in "..ose"
Fig. 5.4,   linear and ring forms of glucose
        Note carbon numbering starts with carbonyl, most oxidized carbon
        Forming ring form is NOT a condensation reaction, note no water released...

Fig. 5.5, sucrose formation, hexose sugars in 6 and 5 sided rings
Condensation Rx, creates a glycosidic linkage, note water production
monosaccharide, disaccharide
Note five sided fructose versus six sided glucose (both are hexoses)
Sucrose is glucose and fructose, note carbon numbers involved
Fig. 5.7, starch vs. cellulose
Alpha vs. Beta glucose; alpha H above, beta H below
Starch alpha 1-4 linkages, cellulose beta 1-4 linkages
These look similar, but the use of alpha glucose versus beta glucose makes very different
end products. We can not digest cellulose's beta 1-4 bonds for instance...
Fig. 5.6, starch structure
also called amylose
alpha 1-4 promotes spiral shape, more open, structurally weaker...
cross linked amylose produces glycogen, a common animal storage form...
Fig. 5.8, cellulose structure
beta 1-4 flips the twist, so it is linear, allows strands to line up and H-bond
promotes strength of cellulose which mass-per-mass is stronger than steel!

Lipids:
Very diverse, grouped by hydrophobicity not by structure
(if it dissolves well in oil it is a lipid)
Typically lots of C and H, little O... has many non-polar covalent bonds
Generally, lack polymers and monomers...

Fig. 5.15,   cholesterol
Sterols: Ringed hydrocarbon, a steroid, OH at one end.
Other examples include our sex hormones.

Fig. 5.12,   fats and fatty acids, saturated vs. unsaturated
Fatty acids: Hydrocarbon chains with alot of C-C and C-H bonds
With carboxyl group at end. Saturated vs. unsaturated, forms kinks in molecule
these kinks interfere with van der Waals interactions
in fats this makes butter distinct from oils...

Fig. 5.11,   triacylglycerol
Fat molecule: Made of three fatty acids and one glycerol.
The acyl groups are the long hydrocarbon chains
condensation reactions used here form ester bonds
but this is NOT polymerization, as can not continue it
A fat molecule is a triacylglycerol. Associate in fat droplets in our cells.

Fig. 5.13,   diacylglycerol, PC
A diacylglycerol is where one of the -OH groups of the glycerol is bound to a
hydrophilic group, called the head group.
These are called phospholipids. Shown here is a Phosphotidylcholine,

note its parts: Pi, choline (head group), diacylglycerol, FAs
Phospholipids are hydrophobic at one end, and hydrophilic at other
Such molecules are said to be amphiphatic.

Fig. 5.13, (Campbell and Reece, 2002)   micelle and membrane
Amphipathic molecules can associate into multi-molecular structures.
Phospholipids come together to form bilayers.
Note this is NOT a polymer, no covalent bonds between the phospholipids.
Uses van der Waals interactions,   Exclude water.
Different regions of the membrane can differ in lipids, producing local domains.

Nucleic Acids:
Nucleotide monomers are added as monophosphates, but brought in initially as triphosphates
phosphoester bond formed by condensation Rx

Fig. 5.27,   Nucleic acids, subunits
Acid, phosphoric acid, phosphate group
Sugar, pentose, ribose or deoxyribose, note carbons #2, 3, 5

Fig. 16.8,   DNA base pair H-bonds
Bases
Pyrimidines   single ring
cytosine, thymine, uracil
Purines   double ring
adenine, guanine AG = chemical symbol for silver, "pure"?
AT: two H bonds between
GC: three H bonds between

Fig. 5.28,   DNA, double helix
single or double stranded, can convert between these two by
making or breaking the weak H-bonds.
A nice example of the use of weak bonds to allow different
structural states...

Fig. 17.14   folding of tRNA
folded RNA, t, r RNAs, mRNAs, and other types exist
Other types derived from nucleic acids include: NAD, FAD, ATP, etc....
Note that some polynucleic acids have binding sites, catalytic sites, and so play more of a role
than just holding information.

Proteins:

The monomers include 20 common types of amino acids
(others exist, these are just the common ones).

Two common suffixes seen in their names:

"...ase"   Such as hexokinase, carboxylase, amylase. These are enzymes.

"...in: Such as myosin, hemoglobin, albumin, keratin. Not always enzymes,
many of these are structural proteins.

Fig. 5.18,   peptide bond

Condensation Reaction, leading to formation of a peptide bond

The fusion of peptides forms the polymer, called a polypeptide.

Notice that this takes very hydrophilic carboxyl and amine groups
and produces a peptide bond that does not ionize.

Still see polar covalent bonds near the peptide bond,
and these allow for hydrogen bonding.

Fig. 5.17,   amino acids

Note typical parts of amino acids.... (DO NOT learn the 20 types...)

Central CH, Terminal amine, Terminal carboxyl

Variable R group: Acid, basic, polar, nonpolar

Levels of protein structure:

Fig. 5.21,   primary protein structure

Primary structure as the order of amino acids in the polypeptide chain.

AA sequence can vary. Note the C terminus and N terminus.

Fig. 5.21,   Secondary structure, beta sheet, alpha helix

Secondary structure is how the primary structure folds up.

We will consider two common secondary structures... (more exist).

Note H bonds, position of R groups

So H-bonds are between the main polypeptide chain, not the side groups.

Fig. 3.9, MBOTC 4th edition, file sec_structure.jpg

Point out various ways to display secondary structures...

Fig. 5.21,   Tertiary structure

Tertiary Structure: How the secondary structures in the polypeptide fold relative to each
other.

How to hold these secondary structures in place?

Intramolecular interactions are used. We have several options here...

H bonds, hydrophobic (i.e. van der Waals)

disulfide bridge, covalent oxidation of -SH HS- to -S-S-

ionic bonds

Note: some of these are fairly weak interactions, weaker than covalent bonds.

So protein shape can be altered fairly easily... that can be good...

Fig. 19.10, (Campbell and Reece, 2002)   DNA binding proteins
     Secondary structure held in final tertiary structure
     Note helices and sheets
     presence of metal ions, cofactors, may play a role in structure
     Last example here is actually two molecules...

Fig. 5.21,   Quaternary structure
Quaternary structure: When several polypeptides associate together to make a final
     protein.
     Interactions between proteins, so now beyond a polymer level...
     Interactions are often non-covalent
          ionic, H-bonds, van der Waals...
          So weak and therefore easily modified...

Fig. 5.24, (Campbell and Reece, 2002)   protein structure
     Review
     The interactions determine the final structure.
     Disrupt the interactions and a protein's function may be altered.

The main point is molecular shapes and properties.
Which class of organic molecules has the highest range of types of interactions?
          (pass out 3X5 cards and get answers)
          Highest?
               next?
               next?
          Lowest?

     Proteins have most, due to the diverse R groups.
     Nucleic acids are next best, the bases are hydrophobic.
     Lipids are diverse, but if are mainly hydrophobic then they can not interact well with
          hydrophilic items.
     Carbohydrates have the same problem, but are just hydrophilic, so can not interact
          well with hydrophobic things.
     So either lipids or carbohydrates could be argued to have the lowest diversity of
          interactions.

So most binding and reactions seen in life are done by proteins and nucleic acids.

Objectives:

Be able to recognize and describe examples of condensation reactions. How do they differ from hydrolysis reactions? Are these condensation reactions a type of redox reaction, why or why not?

Be able to discuss the major characteristics of the four major classes of organic molecules, and the polymers and monomers of three of them. Be able to describe why lipids are not considered to have polymeric forms.

If shown the molecules given in figures 5.5, 5.13, 5.17, or 5.27 be able to recognize hydrophobic or hydrophilic, charged or uncharged, regions of the molecules shown. For those that are monomers, be able to describe what parts of them are used in polymerizations.

Be able to recognize and discuss the role that hydrogen bonding, van der Waals interactions, disulfide bonds, and ionic bonds each play in molecular folding and interactions. Be able to identify bonds involved in determining polymer shapes that are weak, and others that are strong, and relate each to the polymer's functions.

Be able to recognize the difference(s) between items such as those listed below, and know for which class(es) of organic matter each is applicable:
> RNA and DNA?
> A phospholipid and cholesterol?
> Hexoses and pentoses?
> Primary, secondary, tertiary and quaternary structure?
> An alpha-helix and a beta-sheet?
> A purine and a pyrimidine?
> A diacylglycerol and a triacylglycerol?
> A monosaccharide and a disaccharide?
> Ester bond, phosphoester bond, glycosidic bond, and a peptide bond.
> Water soluble and water insoluble molecules.

The "self-quiz" questions #1, 2, 6, and 8 at the end of chapter 5 are worth a look.

Needed overheads and items:

Fig. 5.2, condensation reaction
condens.tif (slidewrite file)
Fig. 5.4,   linear and ring forms of glucose
Fig. 5.5, sucrose formation, pentose and hexose
Fig. 5.7, starch vs. cellulose
Fig. 5.6, starch structure
Fig. 5.8, cellulose structure
Fig. 5.15,   cholesterol
Fig. 5.12,   fatty acids, saturated vs. unsaturated
Fig. 5.11,   triacylglycerol
Fig. 5.13,   diacylglycerol, PC
Fig. 5.13, (Campbell and Reece, 2002)   micelle and membrane
Fig. 5.27,   Nucleic acids, subunits
Fig. 16.8,   Base pairing, H-bonds
Fig. 5.28,   DNA, double helix
Fig. 17.14   folding of tRNA
Fig. 5.18,   peptide bond
Fig. 5.17,   amino acids
Fig. 5.21,   primary protein structure
Fig. 5.21,   Secondary structure, beta sheet, alpha helix
Fig. 3.9, MBOTC 4th edition, file sec_structure.jpg
Fig. 5.21,   Tertiary structure
Fig. 19.10, (Campbell and Reece, 2002)   DNA binding proteins
Fig. 5.21,   Quaternary structure
Fig. 5.24, (Campbell and Reece, 2002)   protein structure

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-<u>Biology</u>. Eighth edition. Chapter 5. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition, Pgs. 68-91. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figures 5.13, 5.24, 19.10. Benjamin Cummings Press. San Francisco, CA.

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002-<u>Molecular Biology of The Cell</u>, 4th edition. Figure 3.9. Garland Science, N.Y., N.Y.

Related Items:

Lipids: The lipids are not static components.
They are actively changed as an organism experiences changes in its environment.

Burkey K.O., R.F. Wilson, R. Wells- 1997-Effects of canopy shade on the lipid
composition of soybean leaves- Physiologia Plantarum 101: 591-598

Some are essential for the normal functioning of our **immune** system cells.

Chun J- 2007-The sources of a lipid conundrum- Science 316: (4/13) 208-210

And the lipid content of the membrane has been shown to alter the activity of proteins in the membrane.

Cornell R.B., R.S. Arnold- 1996-Modulation of the activities of enzymes of membrane
lipid metabolism by non-bilayer-forming lipids- Chemistry and Physics of Lipids
81: 215-227

Carbohydrates:
For more on **starch** structure and sugar **metabolism** see:

Manners D.J- 1985-Starch- Chap. 4, pgs. 149-204, in <u>Biochemistry of storage
carbohydrates in green plants</u>. P.M. Day and R.A. Dixon editors. Academic
Press, London, U.K.
Smith C.J- 1999- Carbohydrate biochemistry. Pages 81-118, chapter 4 in <u>Plant
Biochemistry and Molecular Biology</u>. 2nd edition. P.J. Lea and R.C. Leegood
editors. John Wiley & Sons.

Of course sugars give us sweetness-in-life, and has been studied in plant **nectars**:

Horner H.T., R.A. Healy, G. Ren, D. Fritz, A. Klyne, C. Seames, R.W. Thornburg- 2007-
Amyloplast to chromoplast conversion in developing ornamental tobacco floral
nectaries provides sugar for nectar and antioxidants for protection- American
Journal of Botany 94: (#1) 12-24

Proteins:

Determining protein structure is critical to determine protein **structure**. Here are some recent articles studying the structure of specific proteins that relate that structure to function:

Arkin I.T., H. Xu, M.Ø. Jensen, E. Arbely, E.R. Bennett, K.J. Bowers, E. Chow, R.O. Dror, M.P. Eastwood, R. Flitman-Tene, B.A. Gregersen, J.L. Klepeis, I. Kolossváry, Y. Shan, D.E. Shaw- 2007-Mechanism of Na^+/H^+ antiporting- Science 317: (#5839, 8/10) 799-803

Brown A.E.X., R.I. Litvinov, D.E. Discher, P.K. Purohit, J.W. Weisel- 2009-Multiscale mechanics of fibrin polymer: Gel stretching with protein unfolding and loss of water- Science 325: (#5941, 8/7) 741-744

Huang C-H., D. Mandelker, O. Schmidt-Kittler, Y. Samuels, V.E. Velculescu, K.W. Kinzler, B. Vogelstein, S.B. Gabelli, M. Amzel- 2007-The structure of a human p110α/p85α complex elucidates the effects of oncogenic PI3Kα mutations- Science 318: (#5857, 12/14) 1744-1748

Johnson C.P., H-Y. Tang, C. Carag, D.W. Spelcher, D.E.Discher- 2007-Forced unfolding of proteins within cells- Science 317: (#5835, 8/3) 663-666

Junker J.P., F. Ziegler, M. Rief- 2009-Ligand-dependent equilibrium fluctuations of single calmodulin molecules- Science 323: (#5914, 1/30) 633-637

Lange O.F., N-A. Lakomek, C. Farès, G.F. Schröder, K.F.A. Walter, S. Becker, J. Meiler, H. Grubmüller, C. Griesinger, B.L. deGroot- 2008-Recognition dynamics up to microseconds revealed from an RDC-derived ubiquitin ensemble in solution- Science 320: (#5882, 6/13) 1471-1475

Murzin A.G- 2008-Metamorphic proteins- Science 320: (#5884, 6/27) 1725-1726

Raman S., O.F. Lange, P. Rossi, M. Tyka, X. Wang, J. Aramini, G. Liu, T.A. Ramelot, A. Eletsky, T. Szyperski, A. Kennedy, T. Prestegard, G.T. Montelione, D. Baker- 2010- NMR structure determination for larger proteins using backbone-only data- Science 327: (#5968, 2/19) 1014-1018

Ringe D., G.A. Petsko- 2008-How enzymes work- Science 320: (#5882, 6/13) 1428-1429

Service R.F- 2008-Protein structure initiative: Phase 3 or phase out- Science 319: (#5870, 3/21) 1610-1613

Wu J., S.H.J. Brown, S. von Doake, S.S. Taylor- 2007-PKa type IIα holoenzyme reveals a combinatorial strategy for isoform diversity- Science 318: (#5848, 10/12) 274-279

Zeqiraj E., B.M. Filippi, M. Deak, D.R. Alessi, D.M.F. van Aalten- 2009-Structure of the LKB1-STRAD-MO25 complex reveals an allosteric mechanism of kinase activation- Science 326: (#5960, 12/18) 1707-1711

Just so that you do not get the impression that only L-amino acids are used by life, here is a report of the use of **D-amino acids** by some forms of life.

Lam H., D-D. Oh, F. Cava, C.N. Takacs, J. Clardy, M.A. dePedro, M.K. Waldor- 2009-D-amino acid govern stationary phase cell wall remolding in bacteria- Science 325: (#5947, 9/18) 1552-1555

Nucleic Acids:
The **structure** of **DNA** can vary with tension. And **RNAs** form many complex shapes when they fold.

Bailor M.N., X. Sun, H.M. Zl-Hashimi- 2010-Topology links RNA secondary structure with global conformation, dynamics, and adaptation- Science 327: (#5962, 1/8) 202-206
Mathew-Fenn R.S., R. Das, P.A.B. Harbury- 2008-Remeasuring the double helix- Science 322: (#5900, 10/17) 446-449

There are groups researching the use of DNA for **computation**:

Bar-Ziv R- 2007-DNA circuits get up to speed- Science 318: (#5853, 11/16) 1078-1079
Macdonald J., D. Stefanovic, M.N. Stojanovic- 2008-DNA computers for work and play- Scientific American 299: (#5, Nov.) 84-91
Seelig G., D. Soloveichik, D.Y. Zhang, E. Winfree- 2006-Enzyme-free mucleic acid logic circuits- Science 314: (12/8) 1585-1588

And the uses of DNA in **nanotechnology** has expanded recently. It turns out to be a wonderful material for making all sorts of molecular structures.

Aldaye F.A., A.L. Palmer, H.F. Sleiman- 2008-Assembling materials with DNA as a guide- Science 321: (#5897, 9/26) 1795-1799
Ding B., N.C. Seeman- 2006-Operation of a DNA robot arm inserted into a 2D DNA crystalline substrate- Science 314: (12/8) 1583-1585
Kufer S.K., E.M. Puchner, H. Gumpp, T. Liedl, H.E. Gaub- 2008-Single molecule cut-and-paste surface assembly- Science 319: (#5863, 2/1) 594-596
Seeman N.C- 2004-Nanotechnology and the Double Helix- Scientific American 290: (#6) 64-75
Yin P., R.F. Hariadi, S. Sahu, H.M.T. Choi, S.H. Park, T.H. LaBean, J.H. Reif- 2008-Programming DNA tube circumferences- Science 321: (#5890, 8/8) 824-826

BIO 107   2010
Day 2, Lecture 5, Title: Enzymes and Metabolism
Text Readings:
        Campbell et al. (2008), Chapter 8.

Topics to cover:

        Free Energy and Reaction Cycles
        Altering Non-catalyzed reaction rates
        Enzymes
                Isolating an Enzyme
                Enzyme characteristics
                Regulating and altering enzyme activities

        Free Energy and Reaction Cycles
Energy
        Text tends to refer to "high energy molecules" or the "storage of energy in bonds"
                these are not accurate views, in my view....
        Consider person on side of a hill
                Always higher up the hill? So how can any one spot be high or low in energy?
                        Terms such as high and low only make sense in comparing two sites
                A molecule would be like one site on the hill side, without any comparison.
        Text is trying to attribute "high energy" to one state, it should use a comparison.
Free Energy levels of reactants and products; tendency is to move to lowest energy state
        Fig. 8.14, Energy profile of an exergonic reaction
        activation energy, relate to electronegativity
                Fig. 2.8, electron energy level.
                Gives molecules stability, if not reactions would just happen
                        note error in Fig. 8.6, energy changes but no activation energy barriers!
        net change
                endergonic vs. exergonic reactions, flip sides of each other...
Whether or not a reaction is spontaneous is a different issue from whether or not a molecule is stable
        The text, again, tends to confuse these two issues.
        Return to mountain analogy. Put in local hillock barriers making each point stable.
        Contrast energy change of reaction and stability of molecule,
                note how these are two separate issues
                (The text tends to treat these as the same, but they are not.)
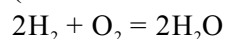
$2H_2 + O_2 = 2H_2O$	Spontaneous reaction to the right.
	Yet there is alot of O_2 in the atmosphere?
$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$	Note wood is made of glucose, and is stable?
	But this reaction is spontaneous.
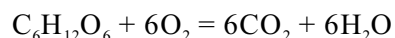
So a negative free energy change is a "spontaneous" reaction.
But the activation energy barrier can make molecules stable.
Even large ΔGs can have stable reactants, IF the activation energy barrier is large.

Altering Non-catalyzed reaction rates

Getting reactions to occur.
Via inorganic chemistry, several options are available.
Consider how each alters the energy levels
concentration changes, adding or removing items.
raising temperature or increasing pressure
altering pH
some of these can denature proteins and destroy cells! so not often used by life
Cells are stuck near Standard Temperature and Pressure (STP)

Enzymes

Why use enzymes? Allows catalysis without extreme conditions.
Not all enzymes are proteins..., nor are all proteins enzymes....
Fig. 8.17, catalytic cycle of an enzyme
How does an enzyme interact with its substrate? Must have a binding site.
?? How can two molecules interact??
H-bonds, ionic interactions, van der Waals, covalent bonds,
?? What part of an amino acid does this interacting?
R group mainly, but not always...
Since these interactions are specific, the binding site is often very specific.
Fig. 3.38, MBOTC, 4th edition, active site
Note involvement of primary to tertiary structure to position the amino acids
note how side groups pull on substrate, substrate here is a nucleotide
Enzyme's influence on reaction cycle: No change in ΔG, but alters the activation barrier.
so it alters the stability of items, but not the net spontaneous direction of the reaction
Fig. 8.15, Enzyme's influence on activation energy
Draw plot of product formation rate vs. [substrate], with and without enzyme
note how this differs from previous plot of free energy vs. reaction cycle stage...

Isolating an Enzyme

Isolating an enzyme, critical for study of its functions. (Note: Not all enzymes are proteins! ;-)
pg. 13, Finean etal., 1978, enzyme isolation
properties of proteins used to separate them, size, charge, binding affinity, etc
For example, can separate some enzymes based on density via centrifugation.
This is tough as have to get the enzyme out of the cell in intact state.
But allows study of enzyme characteristics *in vitro* (i.e. isolated from life) .

Enzyme characteristics

Measuring enzyme characteristics

V_{max} and K_m, draw out plot of reaction rate vs. [S] and identify each.

Concept of saturation, based on limited number of active sites on the enzymes

Altering enzyme concentration leads to changes in reaction rates.

Note that there is ALWAYS some reaction not catalyzed by an enzyme, just slow.

By controling enzyme concentration and activity can alter rate of reaction in cell.

Binding of substrate at the active site changes the shape of the enzyme.

Coupling reactions allows one reaction to influence another reaction

Fig. 8.10, Energy coupling

Note sum of reaction changes.

So enzymes with multiple reactions that interact can couple reactions.

This is vital for synthesis reactions.

Regulating and altering enzyme activities

How to Regulate enzyme activity? This relates to different cells doing different things...

Some ways to alter enzyme activity

Make it work in only certain conditions or in certain places.

Consider an enzyme working outside of cells in the stomach,

versus in a cell's cytosol?

Alter its concentration. (Seen over the time scales of gene expression and growth.)

Make it faster. Degrade it faster. Put lots of it into a small compartment.

It is expensive to make proteins, and it is fairly slow.

Give it, or deny it, substrate.

Change the environment in which the enzyme works (not typical...)

Fig. 8.18, Environmental factors affecting enzyme activity

pH and temperature can alter enzyme activity, but a cell rarely does this.

consider enzymes put in cytosol versus those put in stomach...

Alter the activity of existing enzymes. (A very common method.)

Add or remove something from that enzyme that alters its shape and so

changes the shape of its binding sites.

Methylation, phosphorylation, acetylation, etc...

These are covalent changes, as attach the above by covalent bonds.

Many other types of changes exist...

Add or remove cofactors, ions, etc.

Add or remove subunits. This is altering the quaternary structure.

Some subunits may be needed for activity, others may inhibit the activity.

Use of inhibitors/activators.

Fig. 8.19, inhibitors

Competitive inhibitors, bind at active sites as look somewhat like substrate

Additional binding sites
>This allows coupling of reactions, or binding at one site to influence activity at another
>Allosteric binding sites, are not active sites, are regulatory binding sites
>>Note: The text incorreclty includes covalent modifications as allosteric.
>>>Most consider allosteric control as using non-covalent bonds
>>>>at a distinct binding site...
>Fig. 8.20, Allosteric regulation
>>activators and inhibitors, these bindings could promote or inhibit enzyme activity
>>>change in shape of enzyme alters its ability to catalyze reactions...
>Non-competitive inhibitors, bind at sites other than active sites...
>>This is allosteric regulation.

Final product of a metabolic pathway often acts as a non-competitive inhibitor earlier in the
>pathway.
>Fig. 8.22, Feedback inhibition
>This way get feedback inhibition on the pathway, and so can regulate its activity.
>Fig. 6.1, (Campbell and Reece, 2002)   Complexity of metabolism
>>need feedback mechanisms to be able to control complex metabolic pathways
>>So you expect proteins to have many binding sites,
>>>only a few of which are active sites, many might be allosteric binding sites.

Protein binding with protein can induce cooperative interactions.
>Fig. 8.20, Binding by other proteins
>Multi-protein complexes: Many proteins get together in large complexes to function.
>>See this in many systems. For instance: Hemoglobin, transcription factor
>>>complexes, cytoskeleton.

It all comes down to shape....
>Fig. 3.24A, MBOTC, 4th edition, sizes/shapes of proteins
>Anything that alters the shape of a protein can influence its binding.
>>Binding at one site can alter binding at another, or ability to bind to other proteins.
>>This allows for control of metabolic pathways.

Objectives:

What ultimately determines the rate of a reaction? What determines the net direction of a reaction? What determines whether or not a molecule is relatively stable, compared to whether or not a reaction is spontaneous? Be able to describe the change in free energy as a set of molecules are taken through a reaction cycle to become a set of products when: The reaction is endergonic? The reaction is exergonic?. The reaction is enzyme catalyzed compared to when the reaction is not enzyme catalyzed? The temperature or pressure of the system is raised, compared to the state at a lower temperature or pressure?

(Please note: The text makes references to "high energy molecules" and to the storage of energy "in" bonds or in molecules. I feel it is better to compare energy differences between two states rather than to attribute the energy to one state. Thus, in my view ATP is not a "high energy" molecule with energy stored "in" it, rather the hydrolysis of ATP represents a net release of energy as the energy state of the products is lower compared to that of the reactants. So when you read the text be careful not to fall into the trap of thinking that energy is stored in bonds.)

What are ways that an enzyme can interact with its substrate? Given a specific amino acid and substrate be able to describe several types of bonds, if any, that might be formed between them.

What are two properties a specific enzyme might have that could be used to isolate it from all the other organic molecules in a tissue?

What are V_m and K_m? What information does knowing each of these give you? What are competitive inhibitors and non-competitive inhibitors and how do they differ? What is allosteric regulation of an enzyme's activity, what is needed for this to occur, and how does it differ from covalent modification of an enzyme?

How do enzymes couple reactions? What is the advantage of coupling reactions through the use of enzymes? Be able to describe examples of how an enzyme's ability to couple reactions are be used by cells to convert one form of energy into another form of energy.

Be able to describe several ways in which an enzyme's activity can be altered in a cell.

In chapter 8 of the text, self-quiz questions 4 and 5 are nice.

Needed overheads and items:

Fig. 8.14,   Energy profile of an exergonic reaction
Fig. 2.8,   Electron energy level
Fig. 8.6,   Energy changes (wrong!)
Fig. 8.17,   catalytic cycle of an enzyme
Fig. 3.38, MBOTC, 4th edition, active site, file: prot_active.jpg
Fig. 8.15, Enzyme's influence on activation energy
pg. 13, Finean etal., 1978, enzyme isolation, File: prot_isolation.jpg
Fig. 8.10, Energy coupling
Fig. 8.18, Environmental factors affecting enzyme activity
Fig. 8.20, Allosteric regulation
Fig. 8.19, inhibitors
Fig. 8.22, Feedback inhibition
Fig. 6.1, (Campbell and Reece, 2002)   Complexity of metabolism
Fig. 8.20, Binding by other proteins
Fig. 3.24A, MBOTC, 4th edition, sizes/shapes of proteins, file: prot_shape.jpg

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-An introduction to metabolism. Chapter 8. Pages 142-161. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-Biology, 6th edition, Figure 6.1. Benjamin Cummings Press. San Francisco, CA.

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002-Molecular Biology of The Cell, 4th edition. Figures 3.24A, 3.38. Garland Science, N.Y., N.Y.

Finean J.B., R. Coleman, R.H. Mitchell- 1978-Membranes and their cellular functions. Pg. 13. John Wiley & sons, N.Y.

Related Issues:
For those of you who go on to take biochemistry, a nice overview of **metabolic pathways** and their regulation is given in the following text:

> Salway J.G- 1994-Metabolism at a glance. 97 pages. Blackwell Scientific Publications. Oxford, U.K.

Here is a study that attempts to **model a biochemical pathway**.

> Shinar G., M. Feinberg- 2010-Structural sources of robustness in biochemical reaction networks- Science 327: (#5971, 3/12) 1389-1391

This is a neat historical paper, back-in-the-day when **isolating** even common **enzymes** was truly cutting-edge technology. One study describes a system for studying DNA synthesis in an artificial system was being developed, a vital step in finding DNA polymerase. The other describes a study of an alkaline phosphatase in a manner similar to what is done in the enzyme lab of our course.

> Lehman I.R., M.J. Bessman, E.S. Simms, A. Kornbert- 1958-Enzymatic synthesis of deoxyribonucleic acid. I. Preparation of substrates and partial purification of an enzyme from *Escherichia coli*- Journal of Biological Chemistry 233: (#1) 163-170
> Neumann H., M. Van Vreedendaal- 1967-An improved alkaline phosphatase determination with p-nitrophenyl phosphate- Clinica Chimica Acta 17: (#2) 183-187

Here are some more recent papers that are good examples of **enzyme** isolation, structural determination, and functional characteristics:

> Hrmova M., G.B. Fincher- 2001-Plant enzyme structure. Explaining substrate specificity and the evolution of function- Plant Physiology 125: 54-57
> St. Maurice M., L. Reinhardt, K.H. Surinya, P.V. Atwood, T.C. Wallace, W.W. Cleland, I. Rayment- 2007-Domain architecture of pyruvate carboxylase, a biotin-dependent multifunctional enzyme- Science 317: (#5841, 8/24) 1076-1079
> Ma S.M. J.W-H. Li, J.W. Choi, H. Zhow, K.K.M. Lee, V.A. Moorthie, X. Xie, J.T. Kealey, N.A. DaSilva, J.C. Vederas, Y. Tang- 2009-Complete reconstitution of a highly reducing iterative polyketide synthase- Science 326: (#5952, 10/23) 589-592
> Miller S., B. Taushanjian, A. Oleksy, O. Perisic, B.T. Houseman, K.M. Shokat, R.L. Willams- 2010-Shaping development of autophage inhibitors with the structure of the lipid kinase Vps34- Science 327: (#5973, 3/26) 1638-1642
> Xue Q., E.S. Yeung- 1995-Differences in the chemical reactivity of individual molecules of an enzyme- Nature 373: (Feb 23) 681-683
> Yudushkin I.A., A. Schleifenbaum, A. Kinkhabwala, B.G. Noel, C. Schultz, P.I.H. Bastiaens- 2007-Live-cell imaging of enzyme-substrate interaction reveals spatial regulation of PTB1B- Science 315: (1/5) 115-119

Once we know the **structure** of enzymes then we can determine how to change them to fit our needs. Here are some examples of this:

> Ahn N.G., K.A. Resing- 2005-Lessons in rational drug design for protein kinases- Science 308: (5/27) 1266-1267
>
> Himmel M.E., S-Y. Ding, D.K. Johnson, W.S. Adney, M.R. Nimios, J.W. Brady, T.D. Foust- 2007-Biomass recalcitrance: Engineering plants and enzymes for biofuel production- Science 315: (2/9) 804-807

Here is a report of an **antibiotic** that acts as a **competitive inhibitor** of an essential enzyme activity in bacteria.

> Edwards M.J., R.H. Flatman, L.A. Mitchenall, C.E.M. Stevenson, T.B.K. Le, T.A. Clarke, A.R. McKay, H-P. Fiedler, M.J. Buttner, D.M. Lawson, A. Maxwell- 2009-A crystal structure of the bifunctional antibiotic simocyclinone D8, bound to DNA gyrase- Science 326: (#5958, 12/4) 1415-1418

One study relates **allosteric binding** to changes in **protein shape**. The other considers the use of allosteric sites as possible targets for future **drugs**.

> Hilser V.J- 2010-An ensemble view of allostery- Science 327: (#5966, 2/5) 653-654
>
> Wenner M- 2009-A new kind of drug target- Scientific American 301: (#2, Aug) 70-76

New types of **covalent modifications** of **enzymes** have been found. This offers new ways that the activity of enzymes in pathways could be controlled.

> Bollinger J.M. jr, M.L. Matthews- 2010-Remote enzyme microsurgery- Science 327: (#5971, 3/12) 1337-1338
>
> Jensen L.M.R., R. Sanishvili, V.L. Davidson, C.M. Wilmot- 2010-In crystallo posttranslational modification within a ManG/Pre-methylamine dehydrogenase complex- Science 327: (#5971, 3/12) 1392-1394
>
> Wang Q., Y. Zhang, C. Yang, H. Xiong, Y. Lin, J. Yao, H. Li, L. Xie, W. Zhao, Y. Yao, Z-B. Ning, R. Zong, Y. Xiong, K-L. Guan, S. Zhao, G-P. Zhao- 2010-Acetylation of metabolic enzymes coordinates carbon source utilization and metabolic flux- Science 327: (#5968, 2/19) 1004-1007

Just a reminder that not all **enzymes** are made of proteins! Some enzyme activities are found in other classes of molecules:

> Webb C-H.T., N.J. Riccitelli, D.J. Ruminski, A. Lupták- 2009-Widespread occurrence of self-cleaving ribozymes- Science 326: (#5955, 11/13) 953
>
> Westheimer F.H- 1986-Polyribonucleic acids as enzymes- Nature 319: 534-536

Obviously different cell types have different patterns of metabolism in them. Here is an example of a study of the patterns of **metabolic pathways** seen in different cell types and connecting this to gene expression patterns. They call this a **metabolome**.

Beloqui A., M-E. Guazzaroni, F. Pazos, J.M. Víeites, M. Godoy, O.V. Golyshina, T.N. Chernikova, A. Waliczok, R. Sílva-Rocha, Y. Al-ramahi, V. LaCono, C. Mendez, J.A. Salas, R. Solano, M.M. Vakimov, K.N. Timmis, P.N. Golyshin, M. Ferrer- 2009-Reactome array: Forging a link between metabolome and genome- Science 326: (#5959, 10/9) 252-257

BIO 107   2010
Day 2, Lecture 6, Title: Cells and their parts.

Text Readings: Campbell et al., 2008; Chapter 6.

Topics to cover:

> **Minimal Cell**
> **Surface area/volume issues and Diffusion**
> **Cytosol and Cytoskeleton**
> **Endomembrane System**
> **Endosymbiosis**
> **Extracellular Matrix**
> **Cell-to-cell Connections**

Minimal Cell
> Fig. 6.6x1, *Bacillus polymyxa* (Campbell and Reece, 2005)
?What are the parts of a minimal cell? Think bacterial cell.
> Fig. 6.6, bacterial cell
>> Note: extracellular matrix: peptidoglycan, mucus, etc...
>>> includes: cell wall, capsule, flagella
>> nucleoid has a circular chromosome, holds genetic material
>> cytosol, plasma membrane, ribosomes, pili = fimbriae, etc...
>> ?What is the role of the pili?
> In some cases there ARE reports of bacteria with nuclei (Fuerst, 2005).
> Note also, proteinaceous compartments
>> Tanaka et al 2010 fig 1.jpg
>> Tanaka et al 2010 fig 3.jpg
>> These hold distinct enzyme activities
>> Proteins have ability to control entry/exit of items.
>> (These look to me like viral capsids!)
> Note size, down in micrometer range... under the limits of diffusion...
>> so items in a bacterial cell can diffuse rapidly to other areas of the cell.

Surface area/volume issues and Diffusion
Diffusion
      Bacterial cell about 1 micrometer across, small enough for diffusion
      Eukaryote cells, about 10-100 µm across
            diffusion ok for small items, but cytoplasmic streaming needed for larger ones

Diffusion of viruses in water verus in cell cytosol (Flint et al. 2004).

	Time to diffuse 10 µm distance:	
	In H_2O (seconds)	In cytosol (hours)
Poliovirus	3.85	0.5
Herpes simplex virus	14.6	2.0
Vaccinia virus	35.0	4.9

So the cytosol is not water, but has structures in it that greatly slow down diffusion.
So we have cytoplasmic streaming in large cells as the cell's cytosol is a barrier to diffusion
            of larger items such as big structures like viruses.
Surface area per volume to service
      Issues of entry and waste removal over a given section of plasma membrane.
      How much internal volume can one area of membrane support?
            Fig. 6.8, Geometric relationships
      To keep in touch with outside, use folded shapes.
      To get out of touch with outside (as when go dormant), use circular shape.
            If cell is large, then need to hold cell in certain shape, and get folds in membranes

Cytosol and Cytoskeleton
      Ribosomes, either free in cytosol or associated with the rough ER
            (In bacteria, which membrane would they associate with?)
            Fig. 6.11, ribosomes
            ribosomes are perhaps one of the most ancestral of all organelles?
            site of protein synthesis, and proteins are critical for cell functioning
            Where would ribosomes be located in a prokaryotic cell?
      Proteins act in many functions. Catalysts, binding sites, receptors, and structure.
            Cytoskeletal proteins in the cytosol, and nuclear space.
                gives structural support, shape, force transmission, movement, etc...
            Involved in cytoplasmic streaming and cell shape, etc...
            Note that there are reports of cytoskeleton-like proteins in bacteria (White 2000).
      Review cytoskeletal proteins
            scaffolding proteins and associated motor proteins
                Microtubules made of tubulin subunits, one motor protein is dynein
                Microfilaments are made of actin subunits, one motor protein is myosin
                Intermediate filaments, in the nucleus and other parts of the cell,
                    various types made of various subunits...
            Fig. 6.20, cytoskeleton

Fig. 6.25, cilium bending, dynein (Campbell and Reece, 2005)
Fig. 6.25, cilium binding
Note: Role of dynein as a motor protein here, also cilia can be sites of
sensing local solution. can put receptors in the cilia...
Fig. 6.24, flagellum and cilia
Centrioles and basal bodies
Fig. 6.22, centrioles
Base of cilia and flagella have MT structures to organize them.
These replicate, at molecular level, before cell can divide...
Fig. 6.26, microfilaments and intermediate filaments
Notice how this helps to give the cell surface shape (i.e. a "cyto" - "skeleton")
Fig. 6.27, microfilaments and motility
Motor proteins are ATPases, cleavage of ATP coupled to mechanical motion
typically 1 ATP gives a 5 nm step.
dynein or myosin can bind to a vesicle at one end, and MT or microfilament
at the other
pulls vesicle through cytosol faster than diffusion. Cytoplasmic streaming.
Note how the cytoskeleton can extend from surface of cell into nucleus,
can even alter gene expression!

Endomembrane System
Draw on board how can form nucleus. Infold PM to surround genetic material.
a very complex system of proteins helps membranes interact with cytoskeleton
this produces the folds and changes in shape
Note connections to
ER, Golgi, vesicles, vacuole/lysosome, PM, nuclear envelope
Note membrane flow, new membrane is made at ER, flows out from there...
Fig. 6.16, membrane flow, endomembrane system (Campbell and Reece, 2005)
Secretory pathway allows cell to make and secrete varous proteins.
This is critical for cells in glands.
Nuclear parts
pores, double membrane, chromatin, DNA and proteins, nucleolus, nuclear lamina
Fig. 6.10, nuclear structures
Endosymbiosis
Mitochondria and chloroplast, etc...
note number of membranes and how gotten from bacteria
note similarities to bacteria
Fig. 6.17, mitochondrion
Fig. 6.18, chloroplasts
cytosolic space versus extracellular spaces
chromatin and genetic structure is prokaryotic-like in these organelles
ribosomes are prokaryotic types as well... etc...

Extracellular Matrix

 Made via exocytosis or via membrane transport, puts subunits outside
   then get to assemble into an extracellular matrix
   either cell wall, or a proteinaceous or gelatinous layer...
 Plant, cellulosic cell wall
   Fig. 6.28, plant cell wall
 animal, glycocalyx can include proteins, and sugar groups
   Fig. 6.30, animal ECM
 fungi, polymers of chitinchitin
   fungal_cell.jpg (Fig 1, Griffin 1994, TEM of fungal cells)
 Bacterial, often cell wall is made of peptidoglycans, may have a capsule
   pili (Fimbriae) are protein fibers that are assembled in bacterial ECM
    (Yeates etal. 2007)
   Fig. 6.6
   This provides structure, external anchorage, and protection...
 Notice, when ECM is close to membrane and made of tough polymers
   then cell can have internal turgor pressure.
   Animal cells generally lack this ability.

Cell-to-Cell Connections

 Essential for multicellularity
   Fig. 6.32, animal cell junctions
 surface only: tight junctions can create barriers in extracellular areas
   we use these to keep stomach solutions in the stomach...
 cytosol shared through gap junctions, but membrane is not shared
   can pass small items like sugars or ATP from cell to cell
 Also anchoring to ECM, in structures called hemi-desmosomes
   CAMs, cell adhesion molecules...
   CDs, clusters of differentiation, surface binding proteins...
   So cells can anchor to ECM, such as bones or cartilage
    tension can be transmitted from ECM into cells and through cells
 Examples where there is sharing of both cytosol and membrane
   Fungal gap connections
    Figure 1, Griffin 1994, TEM of fungal cells, File: fungal_cell.jpg
    large enough to allow small organelles to pass between cells!
   plasmodesmata
    note that ER goes does the center, so shares several membranes
    reports of viruses passing through these, as well as mRNA passage
    Fig. 7.8c, Plasmodesmata2.jpg, Salisbury etal (1992)
    Fig. 1.24, Plasmodesmata1.jpg, Taiz and Zeiger (1991)

We've come quite a way from looking at atoms and simple molecules. Now there can be cellular specializations. Many of the items we see here display emergent properties of life since we could not hope to anticipate them from looking at atoms or other lower levels of organization!

With the options we have seen in eukaryotic cells we can get

Connection to other items.
Mobility.
Control of shape can alter interaction with the environment.
Specialization of cell regions, or into cell types.

This can allow multicellularity....

Objectives:

Contrast the structures of a typical bacterial cell versus that of a typical eukaryotic cell. Be able to describe the structure and major function(s) of each cellular part (see figures 6.6 and 6.9 for those that are fair game). What are two differences between plant and animal cells? What are the typical differences between prokaryote and eukaryote cells?

What is an advantage and a disadvantage of a large surface area relative to internal volume? What traits are likely to be seen in a larger or more metabolically active cell? Be able to describe features of eukaryotes cells that act to help the cells overcome the limits of diffusion.

Which eukaryotic organelles contain DNA? How did they get their DNA and how does this relate to the number of membranes each has? What is endosymbiosis? Are there compartments in the cell that lack ribonucleic acids, if so which compartments are they? Which organelles are part of the endomembrane system? Describe the typical flow of membrane; where in the cell is it made and where does it go on its way to the plasma membrane? What is a common feature of the aqueous space within the endomembrane system?

What are several ways in which cells may attach and form junctions with other cells? Which are often used by animal cells and which are often used by plants? What is the purpose of each type of junction? Consider how the use of cell-to-cell connections allows for the organization of cells into tissues with distinct functions.

What is the cytoskeleton? Is it found in prokaryotes or in eukaryotes? Know the major proteins that are a part of the cytoskeleton, the structures they make, and the functions with which they are often associated. What are motor proteins and what do they do?

The extracellular matrix varies broadly. Describe major differences in this as seen in bacteria, animal, plant and fungal cells.

Self-quiz questions #1, 2, 3, 5, 7 at the end of chapter 6 are worth a look.

Needed overheads and items:

Fig. 6.6x1, *Bacillus polymyxa* (Campbell and Reece, 2005)
Fig. 6.6, bacterial cell
Tanaka et al 2010 fig 1.jpg
Tanaka et al 2010 fig 3.jpg
Fig. 6.8, geometric relationships
Fig. 6.11, ribosomes
Fig. 6.20, cytoskeleton
Fig. 6.25, cilium bending, dynein (Campbell and Reece, 2005)
Fig. 6.25, cilium bending, dynein
Fig. 6.24, flagellum and cilia
Fig. 6.22, centrioles
Fig. 6.26, microfilaments and intermediate filaments
Fig. 6.27, microfilaments and motility
Fig. 6.16, membrane flow, endomembrane system (Campbell and Reece, 2005)
Fig. 6.10, nuclear structures
Fig. 6.17, mitochondrion
Fig. 6.18, chloroplasts
Fig. 6.28, plant cell wall
Fig. 6.30, animal ECM
Figure 1, Griffin 1994, TEM of fungal cells,   File: fungal_cell.jpg
Fig. 6.6, bacterial cell
Fig. 6.32, animal cell junctions
Figure 1, Griffin 1994, TEM of fungal cells,   File: fungal_cell.jpg
Fig. 7.8c, Plasmodesmata, Salisbury and Ross (1992), File: plasmodesmata2.jpg
Fig. 1.24, Plasmodesmata, Taiz and Zeiger (1991),   File: plasmodesmata1.jpg

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-A tour of the cell. Chapter 6. Pages 94-124. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-Biology, 7th edition, Figures 6.6, 6.16, 6.25. Benjamin Cummings Press. San Francisco, CA.

Flint S.J., L.W. Enquist, V.R. Racaniello, A.M. Skalka- 2004- Principles of Virology: Molecular biology, pathogenesis, and control of animal viruses- pg. 154. ASM Press. Washington D.C.

Fuerst J.A- 2005-Intracellular compartmentation in Planctomycetes- Annual Review of Microbiology 59: 299-328

Griffin D.H- 1994-Fungal Physiology, 2nd edition. Figure 1. Wiley-Liss Press, N.Y.

Salisbury F.B., C.W. Ross- Plant Physiology, 4th edition. Figure 7.8c. Wadsworth Publishing Co. Belmont, CA.

Taiz L., E. Zeiger- 1991-Plant Physiology. Figure 1.24. Benjamin/Cummings, Redwood City, CA.

Tanaka S., M.R. Sawaya, T.O. Yeates- 2010-Structure and mechanism of a protein-based organelle in *Escherichia coli*- Science 327: (#5961, 1/1) 81-84

White D- 2000-Structure and function- Chapter 1, pgs. 1-36, in, The Physiology and Biochemistry of Prokaryotes. 2nd edition. Oxford University Press, N.Y.

Yeates T.O., R.T. Clubb- 2007-How some pili pull- Science 318: (#5856, 12/7) 1558-1559

Related issues:

Cell shape: Just how varible can a single-celled species be? This one has over a dozen forms. The basis of cell shape is often determined by structures made using actin.

> Burkholder J.M- 1999-The lurking perils of *Pfiesteria*- Scientific American 281: (#2) 42-49
>
> Pollard T.D., J.A. Cooper- 2009-Actin, a central player in cell shape and movement- Science 326: (#5957, 11/27) 1208-1212

Cell-to-cell adhesion: One of these papers is a good overview of cell adhesion in animal cells in general. Another paper examines one cell adhesion protein and its role in nervous tissue. And another notes the uses the adhesive properties of pili by bacteria to enter human brain tissue.

> Coureuil M., G. Mikaty, F. Miller, H. Lécuyer, C. Bernard, S. Bourdoulous, G. Duménil, R-M. Mège, B.B. Wekster, I.A. Romero, P-O. Courand, X. Nassif- 2009- Meningococcal type IV pili recruit the polarity complex to cross the brain endothelium- Science 325: (#5936, 7/3) 83-87
>
> Evans E.A., D.A. Calderwood- 2007-Forces and bond dynamics in cell adhesion- Science 316: (5/25) 1148-1153
>
> Scheiermann C., P. Meda, M. Aurrand-Lions, R. Madani, Y. Yiangou, P. Coffey, T.S. Salt, D. Ducrest-Gay, D. Caille, O. Howell, R. Reynolds, A. Lobrinus, R.H. Adams, A.S.L. Yu, P. Anand, B.A. Imhof, S. Nourshargh- 2007-Expression and function of junctional adhesion molecule-C in myelinated peripheral nerves- Science 318: (#5855, 11/30) 1472-1475

Chloroplasts: Here is some work on how they have been found to send signals to the nucleus in the cell:

> Koussevitzky S., A. Nott, T.C. Mockler, F. Hong, G. Sachetto-Martins, M. Surpin, J. Lim, R. Mittler, J. Chory- 2007-Signals from chloroplasts converge to regulate nuclear gene expression- Science 316: (5/4) 715-719
>
> Zhang D-P- 2007-Signaling to the nucleus with a loaded gun- Science 316: (5/4) 700- 701

Cilia: The cilia are not just motile organelles, they also often have receptors in their membrane, making them primary sites of sensing:

> Christensen S.T., C.M. Ott- 2007-A ciliary signaling switch- Science 317: (#5836, 7/20) 330-331
>
> Rohatgi R., L. Milenkovic, M.P. Scott- 2007-Patched 1 regulated hedgehog signaling at the primary cilium- Science 317: (#5836, 7/20) 372-376

Cytoskeleton: Here are some articles relating with aspects of the cytoskeleton, their accompanying motor proteins, how they connect to transmit force, and how we can use them in nanotechnology:

Alonso M.C., D.R. Drummond, S. Kaln, J. Hoeng, L. Amos, R.A. Cross- 2007-An ATP gate controls tubulin binding by the tethered head of kinesin-1- Science 316: (4/6) 120-123

Aratyn-Schaus Y., M.L. Gardel- 2008-Clutch dynamics- Science 322: (#5908, 12/12) 1646-1647

Bakal C., J. Aach, G. Church, N. Perrimon- 2007-Quantitative morphological signatures define local signaling networks regulating cell morphology- Science 316: (#5832, 6/22) 1753-1756

Mizuno D., C. Tardin, C.F. Schmidt, F.C. MacKintosh- 2007-Nonequilibrium mechanics of active cytoskeletal network- Science 315: (1/19) 370-373

Rodrigues-Martins A., M. Riparbelli, G. Callaini, D.M. Glover, M. Bettencourt-Dias- 2007-Revisiting the role of the mother centriole in centriole biogenesis- Science 316: (5/18) 1046-1050

Schwartz M.A- 2009-The force is with us- Science 323: (#5914, 1/30) 588-589

Shiroguchi K., K. Kinosita jr.- 2007-Myosin V walks by lever action and Brownian motion- Science 316: (5/25) 1208-1212

van den Heuvel M.G.L., C. Dekker- 2007-Motor proteins at work for nanotechnology- Science 317: (#5836, 7/20) 333-336

Vartiainen M.K., S. Guettler, B. Larijani, R. Treisman- 2007-Nuclear actin regulates dynamic subcellular localization and activity of the SRF cofactor MAL- Science 316: (#5832, 6/22) 1749-1752

Wu J.L., G.R. Crabtree- 2007-Nuclear actin as choreographer of cell morphology and transcription- Science 316: (#5832, 6/22) 1710-1711

Endomembrane system: Here are some articles that describe membrane and protein flow through this set of organelles:

Hammond C., A. Helenius- 1994-Quality control in the secretory pathway: Retention of a misfolded viral membrane glycoprotein involves cycling between the ER, intermediate compartment, and golgi apparatus- The Journal of Cell Biology 126: 41-52

McNiven M.A., H.M. Thompson- 2006-Vesicle formation at the plasma membrane and trans-golgi network: The same but different- Science 313: (9/15) 1591-1594

Mizuno M., S.J. Singer- 1993-A soluble secretory protein is first concentrated in the endoplasmic reticulum before transfer to the golgi apparatus- Proceedings of the National Academy of Science (USA) 90: 5732-5736

Endosymbiosis: This is a major issue in eukaryotic cells. Here are some articles relating to it, including some that note how the assembly of proteins in bacterial membranes is similar to how it is done in mitochondria and in chloroplast membranes:

Alcock F., A. Clements, C. Webb, T. Lithgow- 2010-Tinkering inside the organelle- Science 327: (#5966, 2/5) 649-650

Brandvain Y., M.S. Barker, M.J. Wade- 2007-Gene co-inheritance and gene transfer- Science 315: (3/23) 1685

Dagan T., W. Martin- 2009-Seeing green and red in diatom genomes- Science 324: (#5935, 6/26) 1651-1652

Hagan C.L., S. Kim, D. Kahne- 2010-Reconstitution of outer membrane protein assembly from purified components- Science 328: (#5980, 5/14) 890-892

Jeon K.W- 1991-Amoeba and x-bacteria: Symbiont acquisition and possible species change- pgs 118-131 of <u>Symbiosis as a source of evolutionary innovation: Speciation and morphogenesis</u>. L. Margulis and R. Fester editors. MIT Press, Cambridge, MA.

Jeon K.W- 1972-Development of cellular dependence on infective organisms: Microsurgical studies in Ameobas- Science 176: (June 9) 1122-1123

Jeon K.W., M.S. Jeon- 1976-Endosymbiosis in *Amoebae*: Recently established endosymbionts have become required cytoplasmic components- Journal of Cellular Physiology 89: 337-344

Nishihara N., T. Takahashi, T. Kosaka, H. Hosoya- 1996-Characterization of symbiotic algae-free strains of *Paramecium bursaria* produced by the herbicide paraquat- Journal of Protozoology Research 6: 60-67

Nishihara N., T. Takahashi, T. Kosaka, H. Hosoya- 1996-Re-infection of cloned symbiotic algae to algae-free Paramecia- Zoological Science (Tokyo) 13: (Suppl.) 44

Nishihara N., S. Horiike, T. Takahashi, T. Kosaka, Y. Shigenaka, H. Hosoya- 1998- Cloning and characterization of endosymbiotic algae isolated from *Paramecium bursaria*- Protoplasma 203: 91-99

Stroud D.A., C. Meisinger, N. Pfanner, N. Wiedemann- 2010-Assembling the outer membrane- Science 328: (#5980, 5/14) 831-832

Membrane Fusion: These two articles deal with the protein complex (syntaxin) involved in the fusion of vesicles to the plasma membrane in the secretory pathway:

Sieber J.J., K.I. Willig, C. Kutzner, C. Gerding-Reimers, B. Harke, G. Donnert, B. Rammner, C. Eggeling, S.W. Hell, H. Grubmüller, T. Lang- 2007-Anatomy and dynamics of a supremolecular mechano-protein cluster- Science 317: (#5841, 8/24) 1072-1076

White S.H- 2007-Crowds of syntaxins- Science 317: (#5841, 8/24) 1045-1046

Mitochondria: Turns out some factors in the mitochondria influence life span in animals in a way that might be linked to oxidative stress.

Hajnóczky G., J.B. Hoek- 2007-Mitochondrial longevity pathways- Science 315: (2/2) 607-609

Pinton P., A. Rimessi, S. Marchi, F. Orsini, E. Migliaccio, M. Giorgio, C. Contursi, S. Minucci, F. Mantovani, M.R. Wieckowski, G.D. Sal, P.G. Pelicci, R. Rizzuto- 2007- Protein kinase Cβ and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66[Shc]- Science 315: (2/2) 659-663

Wallace D.C., J.M. Shoffner, I. Trounce, M.D. Brown, S.W. Ballinger, M. Corral-Debrinski, T. Horton, A.S. Jun, M.T. Lott- 1995-Mitochondrial DNA mutations in human degenerative diseases and aging- Biochemica et Biophysica Acta 1271: 141-151

Nucleus: Some recent articles about aspects of the nucleus:

Hurtley S.M., E. Pennisi- 2007-Journey to the center of the cell- Science 318: (#5855, 11/30) 1399

Lim R.Y.H., B. Fahrenkrog, J. Köser, K. Schwarz-Herion, J. Deng, U. Aebi- 2007-Nanomechanical basis of selective gating by the nuclear pore complex- Science 318: (#5850, 10/26) 640-643

MelčáK I., A. Hoelz, G. Blobel- 2007-Structure of Nup58/45 suggests flexible nuclear pore diameter by intermolecular sliding- Science 315: (3/23) 1729-1732

Terry L.J., E.B. Shows, S.R. Wente- 2007-Crossing the nuclear envelope: Hierarchical regulation of nucleocytoplasmic transport- Science 318: (#5855, 11/30) 1412-1416

Travis J- 2007-Return of the matrix- Science 318: (#5855, 11/30) 1400-1401

Trinkle-Mulcahy L., A.I. Lamond- 2007-Toward a high-resolution view of nuclear dynamics- Science 318: (#5855, 11/30) 1402-1407

Stewart C.L., K.J. Roux, B. Burke- 2007-Blurring the boundary: The nuclear envelope extends its reach- Science 318: (#5855, 11/30) 1408-1412

The **cytosol** is not a homeogenous solution. Rather it has distinct regions and many different types of **protein complexes**, some of which are recognized as organelles. Some argue that **prions** are types of aggregates that have gone bad. In other cases the cytosolic differences are associated with differences in the local membranes, this can produce local compartments of cytosol.

Dorn II G.W- 2010-Refugee receptors switch sides- Science 327: (#5973, 3/26) 1586-1587

Kang S., T. Douglas- 2010-Some enzymes just need a space of their own- Science 327: (#5961, 1/1) 42-43

Miller G- 2009-Could they all be prion diseases?- Science 326: (#5958, 12/4) 1337-1339

Nikolaev V.O., A. Moshkov, A.R. Lyon, M. Miragoli, P. Novak, H. Paur, M.J. Lobse, Y.E. Korchev, S.E. Harding, J. Gorelik- 2010-β_2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation- Science 327: (#5973, 3/26) 1653-1657

Prokaryotes: Here are some texts and articles for more information on bacterial cells:

Bai F., R.W. Branch, D.V. Nicolau jr., T. Pilizota, B.C. Steel, P.K. Maini, R.M. Berry- 2010-Conformational spread as a mechanism for cooperativity in the bacterial flagellar switch- Science 327: (#5966, 2/5) 685-689

Cole S.T., I. Saint-Girons- 1999-Bacterial genomes - all shapes and sizes- Chapter 3, pgs. 35-62, in, Organization of the prokaryotic genome- R.L. Charlebois editor. American Society for Microbiology, Washington D.C.

Leblond P., B. Decaris- 1999-Unstable linear chromosomes: The case of *Streptomyces*- Chapter 14, pgs. 235-261, in, R.L. Charlebois editor, Organization of the Prokaryotic Genome. American Society for Microbiology. Washington D.C.

White D- 2000-The Physiology and Biochemistry of Prokaryotes. 2nd edition. Oxford University Press, N.Y.

BIO 107   2010
Day 3, Lecture 7, Title: Membrane Structure and Function

Text Readings: Campbell etal. (2008), Chapter 7 (but ignore pgs. 133-134), and see pgs. 768-770, 954-955.

Topics to cover:

> **Conservation of Energy**
> **Membrane Structure: Fluid-Mosaic Model**
> **Osmosis**
> **Transport Systems**

Most biological membranes are 6-12 nm wide.   A nice scale bar of cellular/molecular structures...

Conservation of Energy
> Life is able to transduce/transform energy. So we are energy transducers.
>> Our text does a good job on concentration gradients, but does a poor job on
>>> energy in electrical gradients and other forms.
> Need a membrane to maintain concentration and electrical gradients.
> Need enzymes to change molecules and tie these changes to other energy forms.
>> Changes in shape allows transduction of energy.
>> Ex: Myosin couples energy from ATP hydrolysis to change protein shape
>>> This change in protein shape allows generation of force.
>>> Producing pressure....
> Concept of conservation of energy   (slidewrite file: energy.jpg)
> Total energy = energy in .... + ...
>> Pressure, leads to bulk flow   ex: heart pumping
>> Concentration, leads to diffusion   ex: glucose moving down this gradient
>> Bulk electric fields, lead to electrophoresis of ions   ex: action potentials
>> Atomic electronegativity, leads to chemical reactions   ex: chemical energy
>> Electromagnetic packets, heats atoms/molecules   ex: light
>> Heat is least useful form of energy,
>>> other forms are "Free Energy" as can be used to do work.
> Energy use converts energy from useful "free energy" forms to less useful heat...

Membrane Structure: Fluid-Mosaic Model
Menbranes show semi-permeability   (fig. 7.2)
> hydrophobic center, with amphipathic molecules
>> Recall: What does amphipathic mean?
> What are examples of molecules that are amphipathic?   (fig. 7.7)
> In membrane note: phospholipids, sterols, proteins, integral vs. peripheral proteins...

Fluid-mosaic model   (fig. 8.2; Campbell and Reece, 2002)
            Major change from previous model was to put proteins spanning the membrane...
      How fluid?   (fig. 7.5)
            The lipids do move. But this is NOT what what "fluid" refers to in the model.
                  Note: Effect of fatty acid saturation, and rarity of "flip-flop"
            (fig. 7.6)
      Note experiment by Frye and Edidin (1970), used by Singer and Nicolson (1972)
                  fused two cells with distinctly labeled proteins in membrane
                  observed the membrane proteins move over time
            proteins can move in plane of membrane, so display fluidity.
            If all proteins in membrane are fluid, then membrane would be homogenous?
      How a mosaic
            (fig. 7.7, membrane)
            Note protein connections, so can be held in plane of membrane
            Note advantages of positioning membrane proteins at different points...
            integral vs. peripheral membrane proteins
      Note these properties (fluid-mosaic) refer to proteins, not to lipids....

Osmosis
      Diffusion of water down its energy gradient across a membrane
            can get water concentration gradient balanced by pressure gradient
            energies in each of these, so back to conservation of energy...
            total energy gradient of water across a membrane =
                  (energy in water concentration gradient) + (energy in pressure gradient)

      What if a concentration gradient of sucrose and water across a membrane?
            Water is smaller, so moves faster
            sucrose does cross, it just takes hours to days
            water crosses fast, in seconds - minutes
            So over short term we can assume sucrose does not move
                  and see the consequence of water movement.
            This is osmosis.
      Two sets of terms that conpare two solutions...
            Osmotic terms: Hyper-, Hypo-, Iso- osmotic
                  differences in solute concentration, and so in water concentration.
                  If there is no pressure gradient, then this tells you the direction of osmosis

            Tonic terms: Hyper-, Hypo-, Iso- tonic   (I will mainly ignore these terms)
                  differences in energy state of the water due to both
                        concentration and pressure factors.
                  Note on pg. 133-134 the text incorrectly refers to tonic terms when it
                        should be using the osmotic terms....

So what are these concentration gradients like in reality?

(overhead: = water_conc.jpg)

Concentration gradients of water and sucrose for various internal sucrose solutions relative to pure external water (Weast 1975; pg. D-261).

Internal concentration of Sucrose		Internal concentration of water		Concentration gradient relative to external pure water (Molar)	
(Molar)	(g/L)	(Molar)	(g/L)	Water	Sucrose
0	0	55.5	1000.0	0	-
0.015	5.0	55.2	995.2	0.3	0.015
0.030	10.0	55.1	991.9	0.4	0.030
0.060	20.1	54.7	985.7	0.8	0.060

As water moves into enclosed space, it generates a pressure gradient

So energy in concentration gradient becomes balanced by energy in pressure gradient

Fig. 36.8   U tube examples

note consequence of pressure, with/without cell wall

Fig. 36.9, water relations in cells

Consider here the consequences of osmosis on the cell in pure water.

Its internal water is at the same energy level at that of the water (i.e. isotonic)

But its cytosol has a water concentration much lower than that of the pure water (i.e. hyperosmotic)

The energy difference due to the solute concentration differences is balanced by the energy present in the pressure difference.

The cell is transducing energy types across its membrane by osmosis!

Life can use an osmotic gradient to generate a pressure gradient.

(Note: The text mixes up the tonic and osmotic terms on pgs. 133-134, and it tends to ignore the influence of pressure on osmosis. See fig. 44.2 and pgs. 954-955 of the text for a more proper description of osmotic terms. And see pgs. 768-770 of the text for a better description of osmosis that takes both concentration and pressure factors into account.)

Transport Systems

So water moves fairly freely. Living cells often do not try to actively move water.

Instead move solutes and let the water follow by osmosis...

Transport systems across a membrane?

Transmembrane proteins

(fig. 7.8) Note: alpha helix, where would it be hydrophobic? Hydrophilic?

What membrane proteins do (fig. 7.9)

Note options for their functions.   Will focus here mainly on transport...

Which of these functions are not common in single-celled organisms?

Passive vs. active transport (fig. 7.17)
    (Put on board and fill in under as go)
    Passive: uses existing energy gradient across the membrane.
        can use concentration gradient, and/or electrical gradient....
    Active: couples energy from some other source (i.e. chemical energy, light
        energy) to create a larger energy ACROSS the membrane.

Transport proteins (fig. 7.15)
    Passive transport
        Channels vs. carriers
            fast vs. slow, millions vs. hundreds per second
            Channels turn on/off on millisecond time scale.
            Carriers often act continuously.
    Active transport
        H^+-ATPase (fig. 7.18)
        note change in concentration and electrical field generated across membrane
        energy change in ATP hydrolysis to ADP and Pi is larger
        so is an energy coupling system, found in plants, animals, fungi, prokaryotes....
        Many other types of pumps exist
            Ca^{+2}-pumping ATPase, Na^+/K^+-ATPase, etc...
        All use energy from some source to create an energy gradient across the membrane.

    Generation of electrochemical gradient allows co-transport (fig. 7.19)
        such as antiports, symports
            these are passive transport systems
        energy in one gradient can be coupled to movement of another item

    Consider how this can drive uptake of selected items.

    Consequences of transport. (HMS_cell; Finean etal., 1978)
        So can use a pump to create electro-chemical gradients across a membrane.
        This can allow passive flow through
            channels, and via symports.
        This involves energy transduction,
            and results in the contents of the cell being distinct from outside
            and allows the cell to regulate flow across the membrane.
        (Obviously membrane proteins have other functions...)

Membranes allow:
    Definition of distinct soluble compartments, self versus not self, in versus out.
    Where the cell interacts with the exterior.
    Holds energy gradients, in concentration or charge differences
        So a site of energy transductions...

If time...

        Review membrane flow and how proteins get to PM, recall endomembrane system
            (fig. 7.10)

        Review endocytosis, for digestion inside of cell.
            (fig. 7.20)
            this can take items to internal food vacuole.

        Possibility of transcytosis, across cell layer entirely....
            an issue at the tissue level, such as in digestive tract...
            this can allow items to cross a layer of cells without crossing a membrane

Objectives:

Be able to describe the important structural and functional aspects of the Singer-Nicholson Fluid-Mosaic model. How do integral membrane proteins differ from peripheral membrane proteins? What must those proteins that take part in a mosaic be doing that the fluid proteins do not?

What are types of energy normally converted by organisms from one energy form to another? What class of molecules is typically used by cells to do this conversion? Be able to give examples of such energy conversions.

Define osmosis and given sufficient information be able to determine which way water will flow across a membrane. Energy in what forms often influences the movement of water across biological membranes? Be able to describe two solutions in terms of their relative osmolarity (i.e. hyperosmotic, hypoosmotic, or isoomotic). Describe how even though a cell is hyperosmotic to surrounding pond water (i.e. the pond water is hypoosmotic relative to the cell's contents), yet the water in the cell and the pond can be at the same energy level.

How are transport systems able to move items into the cell up their concentration gradient? What properties of molecules influence their ability to cross a biological membrane?
How do ion channels differ from ion pumps? What is the difference between active and passive transport? Be able to describe examples of both types of transport. Describe an example of a co-transport system. How do symport co-transport systems differ from antiport co-transport systems?

What are some other functions that membrane proteins address for cells other than that of physical transport of items across the membrane?

Self-quiz questions #2 and 5 of chapter 7 are worth looking at.

Needed overheads and items:

slidewrite file: lect7a.tc = energy.jpg
Fig. 7.2, Membrane hydrophobocity
Fig. 7.7, Fluid mosaic model, and ECM, cytoskeleton
Fig. 8.2, (Campbell and Reece, 2002)
Fig. 7.5, Membrane fluidity
Fig. 7.6, Membrane protein and membrane fluidity
Fig. 7.7, Fluid mosaic model, and ECM, cytoskeleton
Fig. 36.8, Osmosis, U tube examples
Water conc. gradients in sucrose solution, water_conc.jpg (Weast, 1975)
Fig. 36.9, water relations of a cell
Fig. 7.8, Transmembrane protein, alpha helix
Fig. 7.9, Functions of membrane proteins
Fig. 7.17, Passive and active transport
Fig. 7.15, Facilitated diffusion
Fig. 7.18, H^+ pump
Fig. 7.19, Cotransport system
HMS_cell.jpg, (Finean et al., 1978) transport systems
Fig. 7.10, Membrane flow and membrane protein delivery
Fig. 7.20, endocytosis

Handout: Osmosis and energy figures....

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BIO 107   2010
Day 3, Lecture 8, Title: Respiration I
Text Readings: Campbell et al. (2008), Chapter 9, pgs. 162-172, 177-182.

Topics to cover:

>   **Overview**
>   **Chemical Energy**
>   **Glycolysis**
>   **Fermentation**
>   **Pyruvate Import**
>   **Citric Acid Cycle**
>   **Regulation and Various Food Sources**

Overview   general reactions and bioenergetics
>   Fig. 9.6,   Oxidative resp. overview
>   Stages: We will cover certain common stages.
>   Glycolysis
>   (Fermentation)
>   Pyruvate import
>   Critic Acid cycle (TCA cycle, Kreb's cycle...)
>   Electron Transport Chain
>   Chemiosmosis
>   We will get up through the TCA cycle today, and do the rest tomorrow
>   Students should know substrates/products for each stage, and
>   net for what goes in and what comes out, and locations of each...
>   Oxidative vs. substrate-level phosphorylation mechanisms for ATP production...
>   Two general means of making ATP
>   Today will cover substrate-level phosphorylation mechanism.

Chemical Energy   What is it?
>   On board example:
> $CH_4 + 2O_2 = 2H_2O + CO_2$
>   Put energy plot on board. Reactant energy of formation = 4.40 aJ
>   Product energy of formation = -5.46 aJ. Note resulting ΔG of -1.14 aJ.
>   The free energy change indicates the SPONTANEOUS direction of the reaction,
>   it does NOT indicate if the reactants are stable.
>   Stability is based on the activation energy barrier.
>   Bond energies.
>   Fig. 9.3, methane and oxygen = CO_2 and water
>   Energy is NOT stored IN bonds, since making bonds releases energy
>   However, changing bonds can produce an energy change
>   and that is how we access chemical energy.
>   Cotterill 2002 respiration example.jpg   Calculation of relative bond energies
>   Cotterill 2002 Table 3-4.jpg   Energies needed to break various bond
>   1 aJ = 1 attoJoules = 1 X 10^{-18} joules of energy
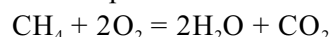
Cotterill 2002 Fig 3-7.jpg   Energies needed to break bonds in glucose
        Note can compare initial and final bonds and calculate rough ΔG estimate
Tell students to compare bond energies of:
        Two C-C bonds versus one C=O double bond.
        One C-H bond versus one O-H bond.
Note the change in net energy as oxidize the carbons and make water.
        this is the chemical energy we access during respiration.
Examples: (see handout) Oxidation of carbon from different redox states.

$CH_4 + 2O_2 = CO_2 + 2H_2O$	**-1.14 aJ**
$CH_3OH + 3/2O_2 = CO_2 + 2H_2O$	**-0.89 aJ**
$H_2CO + O_2 = CO_2 + H_2O$	**-0.57 aJ**
$HCOOH + 1/2O_2 = CO_2 + H_2O$	**-0.32 aJ**
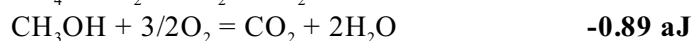
        So we can estimate the free energy differences (this ignores entropy).

Identifying a redox reaction, and those that are not redox reactions.
        Several ways to define redox: Gain/loss of electrons, or of bonds to O or H.
        Oxidized carbon has tighter electrons than reduced C.
        (Carbon redox overhead, slidewrite file: = carbon_redox.jpg; handed out earlier)
        Note that as go from C-C or C-H to C-O or C=O get net release of energy.
           so carbon redox states have an inherent energy gradient.
        We use the energy from these bond changes to drive other reactions.
        As oxidize carbon must put the Hs removed somewhere,
           So need H acceptors (acceptors of H^+ and e^{-1})
        NAD as a hydrogen acceptor:
        Fig. 9.4,   NAD ox and reduced
        note changes in bonds, note how this is a redox change for the carbons
        So if oxidize one molecule then have to reduce another,
           so as oxidize the carbons things like NAD get reduced to NADH

Phosphate bonds
        Fig. 8.9,   ATP cleavage
        Avoid the trap of thinking of ATP as a "high energy" molecule
           just sitting there ATP does not hold energy in its bonds
           it is the conversion of its bonds to other bond types that accesses energy.
        Substrate level phosphorylation
           Fig. 9.7, substrate level phosphorylation
           here take Pi from an organic molecule, and donate it to ADP, creates ATP.
           energy change of dephosphorylation of the organic donor
              must be greater than the energy change of ATP formation
              this drives ATP formation forward.

P$_i$ bond changes are often used in energy transduction

    Show energy shifts in this reaction.

    Fig. 2.75, MBOTC, phosphatebonds.jpg

        Note in this figure, alcohol, amine, acid, enol

    Note that some P$_i$ groups have negative charges, these repell each other

        so activation energy barrier is low already

so can acess this energy by cleavage the bonds in ATP

    and making new bonds that give off more energy

P-glucose = stable,   ATP = stable

    so phosphorylated molecules can be stable.

creatine phosphate, 1,3-bisphosphoglycerate, Phosphoenolpyruvate

    all are stable, but hydrolysis givens higher free energy changes

So ATP hydrolysis is not the largest nor smallest free energy change.

    it is intermediate. (Goldilocks Principle?)

    But do not confuse this energy change with molecular stability

Can couple exergonic reactions to endergonic ones via enzymes

    This supplies free energy to drive endergonic reactions.

        In most of respiration the endergonic reactions are:

            Reduction of NAD to NADH

            Reduction of FAD to FADH$_2$

            Phosphoylation of ADP to ATP

Glycolysis

    Fig. 9.8, Overview of glycolysis

        Note net summary of glycolysis per glucose

        1 glucose becomes 2 pyruvate

        2 ADP + P$_i$ become 2 ATP + 2 H$_2$O

            this is substrate level phosphorylation...

            since these phosphate groups are donated from organic molecules

        2 NAD$^+$ plus 4H becomes 2 NADH

How to tell if there is a net change in redox state of carbons in a pathway:

    First for entire glycolysis pathway:

    Fig. 9.9, Glycolysis reactions

        Note oxidation state of glucose   (at level of CH$_2$O)

            one glucose molecule has

            7 C-O bonds, 7 C-H bonds

        Note oxidation state of pyruvate   (has an acid, carbonyl, methyl group)

            make two of these so have to double it to balance the number of Cs

            5 C-O bonds, for two = 10;   3 C-H bonds, for two = 6

    Thus we go in glycolysis from 7(C-O) to 10(C-O) bonds, net oxidation

        and we go from 7(C-H) to 6(C-H) bonds, also an oxidation

    Notice that the H's lost are picked up by NAD.

Now consider just one reaction in this pathway:

    Look at G-3-P to 1,3-BPGA (Rx #6)

        G-3-P

4 C-O bonds; 4 C-H bonds
1,3-BPGA
5 C-O bonds; 3 C-H bonds
So this is a net oxidation of the carbons,
note that this reaction is coupled to reduction of NAD
Compare to isomerase (Rx #5)
no net change in oxidation here. (students should confirm this...)
So in glycolysis the partial oxidation of the carbons is used to power:
ATP production
We also get the Hs moved to NAD, forming NADH
Fig. 9.8, Overview of glycolysis
Notice, no CO_2 made and no O_2 consumed
We also get pyruvates as end products...

Fermentation
If no oxygen gas (i.e. anaerobic), then pyruvate does not enter mitochondria.
Fig. 9.19, oxidative/non-oxidative switch
so the importation step is under tight regulation...
Fig. 9.18, Fermentation
Problem is that due to glycolysis the cell
accumulates NADH and accumulates pyruvate (hard to export)
Fermentation acts to solve these two problems
Fermentation recovers NAD^+ and forms an easily exported product(s)
In humans, go through lactate recovery.
Net for fermentation
NADH becomes NAD^+
Pyruvate becomes ethanol and CO_2 or lactate
recovery of NAD^+ allows glycolysis to continue, giving more ATP
So this allows glycolysis to continue, and not suffer from a lack of NAD^+
finds a home for the Hydrogens taken from the sugar
and it allows the removal of the pyruvate so this does not accumulate.

Pyruvate Import
If oxygen gas is present (i.e. aerobic), pyruvate goes to mitochondria.
Fig. 9.10, Pyruvate import, pyruvate dehydrogenase
Pyruvate import, after glycolysis, but before citric acid cycle
So a linker step, but not really part of either pathway?
A major point of regulation.
The pyruvate dehydrogenase complex is a membrane spanning complex
it does all of the steps shown in this figure.

4

These reactions have a large energy change, so are fairly irreversible
Note this occurs at the inner mitochondrial membrane, and
see first CO_2 formation
Net:
Pyruvate and CoA becomes CO_2 and acetyl CoA
NAD^+ becomes NADH; again picking up Hs
(Students may wish to confirm that the carbons have been oxidized...)

Critic Acid Cycle   (also called Kreb's cycle, Tricarboxylic acid cycle, TCA cycle....)
This is done in the Matrix of mitochondria
Fig. 9.11, Citric Acid cycle overview
Note that this pathway starts with acetyl CoA, not Pyruvate
This figure incorrectly starts TCA with pyruvate... (an error in this figure)
This pathway will make NADH, $FADH_2$, CO_2, and ATP.
Net:
1 Acetyl CoA becomes 2 CO_2 and 1 CoA; an oxidation of the Cs
1 FAD becomes 1 $FADH_2$ reductive
3 NAD^+ become 3 NADH   reductive
1 GDP and P_i becomes 1 GTP (converted to ATP) and water
ATP formation is endergonic.
This is the first time we have seen FAD, so consider its structure
overhead: FADH, Racusen (1977), FADH.jpg
Note redox changes that occur here (What class of organic molecules is this?)

Fig. 9.12,   TCA cycle reactions
Students should be able to find redox Rxs here,
and identify which are not redox reactions? Where are we in the cell?
Do not memorize the steps, but learn the net results of each pathway we cover,
be able to list all the substrates and products for each,
and know the cellular compartment in which each pathway typically occurs...
For students: What is result of one glucose going to end of TCA cycle? List products.

Regulation and Various Food Sources
(If time, cover regulation and food entry, if not assign for them to consider from reading...)
Regulation
Generally, steps earlier in a process is the point of regulation
Here the enzyme phosphofructokinase is one regulator point
it has allosteric binding sites...
Fig. 9.21, control of cellular respiration
Low ATP concentration means high AMP concentration
binding of AMP stimulates phosphofructokinase activity
High ATP concentration
when bound this inhibits phosphofructokinase activity
High citrate concentrations
when bound this inhibits phosphofructokinase activity

Draw enzyme
  must have binding sites for
    Substrates: ATP and F-6-P   must bind at an active site
    Allosteric sites: ATP, AMP, citrate
How food feeds in to respiratory pathways...
    (If have excess ATP can run this backwards and make fat, AAs, etc...)
Fig. 9.20, Catabolism of food
Fats go to glycerol and fatty acids, note entry point of glycerol and fatty acids
Starch goes to monosaccharides, converted to glucose or fructose for use.
Proteins go to mino acids that enter at several points
    Note, the amine group is striped off, and put into urea in humans.

Next we cover how to deal with all these accumulating reduced molecules: $FADH_2$ and NADH.

Objectives:

Be able to identify the starting and ending points of: glycolysis, fermentation, pyruvate import, and the citric acid cycle. For each of these processes be able to describe the pathway in terms of substrates consumed and products produced and the final net result. Know where and under what conditions each is typically done in the cell. Be able to rank different reactions in terms of their relative energy changes.

What are redox reactions? Given two carbon compounds be able to identify which is more oxidized and which is more reduced by examining the redox state of each of their carbons. If shown the molecules involved be able to identify whether a reaction is or is not a redox reaction. When given sufficient information about a reaction be able to determine whether or not it is a spontaneous reaction, and whether or not the reactants are stable under the stated reaction conditions.

Phosphate bonds play an important role in energy transduction in cells. Consider examples from the intermediates in glycolysis that would be able to promote the formation of ATP and those that would not. What is substrate-level phosphorylation? If shown the molecules involved in one reaction of the respiratory pathways be able to identify whether it is directly involved in substrate-level phosphorylation.

When does a cell shift to fermentative respiration? With what problems does fermentation deal and what benefits does it provide?

What compartments of the cell are involved in the various stages of cellular respiration? How does compartmentation of biochemical pathways benefit eukaryotes? Where in an aerobic bacterium would these pathways be carried out?

Understand how different sources of carbon molecules from various types of food can be funneled into glycolysis.

What are the ways in which feedback can play a role in regulating cellular respiration? At what point(s) in the process does this regulation typically take place?

Self-quiz questions #1, 3, and 9 of chapter nine are worth a look.

Needed overheads and items:

Fig. 9.6, Oxidative resp. overview
Fig. 9.3, methane and oxygen = CO_2 and water
Cotterill 2002 respiration example.jpg   Calculation of relative bond energies
Cotterill 2002 Table 3-4.jpg   Energies needed to break various bond
Cotterill 2002 Fig 3-7.jpg   Energies needed to break bonds in glucose
Carbon redox overhead,   carbon_redox.jpg
Fig. 9.4,   NAD ox and reduced
Fig. 8.9,   ATP cleavage
Fig. 9.7, substrate level phosphorylation
Fig. 2.75, Phosphate bond energies, MBOTC   File: phosphatebonds.jpg
Fig. 9.8, Overview of glycolysis
Fig. 9.9, Glycolysis reactions
Fig. 9.8, Overview of glycolysis
Fig. 9.19, oxidative/non-oxidative switch
Fig. 9.18, Fermentation
Fig. 9.10, Pyruvate import
Fig. 9.11, TCA cycle overview
FADH, Racusen (1977), FADH.jpg
Fig. 9.12, TCA cycle reactions
Fig. 9.21, control of cellular respiration
Fig. 9.20, Catabolism of food

Handout: Cotterill jpgs

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002-<u>Molecular Biology of the Cell</u>, 4th edition. Fig. 2.75, pg. 98. Garland Science Press, N.Y.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Cellular respiration. Harvesting chemical energy. Chapter 9. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Cotterill R- 2002-<u>Biophysics. An Introduction</u>. Pgs. 34-35. John Wiley & Sons. West Sussex, England.

Cram J.M., D.J. Cram- 1978-<u>The Essence of Organic Chemistry</u>. Pg. 35. Addison-Wesley Publishing Co., Reading, MA.

Racusen D- 1977-<u>A pocket guide to bio-organic structures</u>- University of Vermont Press. Camden, Maine.

Related issues:

A set of **reviews** of, and studies of **respiration** in, mitochondria:

> Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walker- 2002-Energy conversions: Mitochondria and chloroplasts. Chapter 14, pgs. 767-829, in <u>Molecular Biology of the Cell</u>. 4th edition. Garland Science, N.Y., N.Y.
>
> Han H-X., O. Khalimonchuk, M. Schraders, N. Dephoure, J-P. Bayley, H. Kunst, P. Devilee, C.W.R.J. Cremers, J.D. Schiffman, B.G. Bentz, S.P. Gygi, D.R. Winge, H. Kremer, J. Rutter- 2009-*SDH5* a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma- Science 325: (#5944, 8/28) 1139-1142
>
> Miller A.H., L.J. Sweetlove, P. Giegé, C.J. Leaver- 2001-Analysis of the *Arabidopsis* mitochondrial proteome- Plant Physiology 127: (#4) 1711-1727
>
> Panov A., A. Scàrpa- 1996-Mg^{+2} control of respiration in isolated rat liver mitochondria- Biochemistry 35: (#39) 12849-12856

The **outer membrane** of mitochondria have large pores that make them highly permeable to small organic molecules.

> Aljamal J.A., G. Genchi, V. DePinto, L. Stefanizzi, A. DeSantis, R. Benz, F. Palmieri- 1993-Purification and characterization of porin from corn (*Zea mays* L.) mitochondria- Plant Physiology 102: 615-621

The genetic material in mitochondria is often useful in determining relationships between species as a part of studies of the **evolution** of species. Also below is a study of the **movement of genes** from the mitochondria to the nucleus:

> Aubert J., M. Solignac- 1990-Experimental evidence for mitochondrial DNA introgression between *Drosophila* species- Evolution 44: (#5) 1272-1282
>
> Duff R.J., D.L Nickrent- 1999-Phylogenetic relationships of land plants using mitochondrial small-subunit rDNA sequences- American Journal of Botany 86: (#3) 372-386
>
> Gilbert M.T.P., L.P. Tomsho, S. Rendulic, M. Packard, D.I.Drautz, A. Sher, A.Tikhonov, L. Dalén, T. Kuznetsova, P. Kosintsev, P.F. Campos, T. Higham, M.J. Collins, A.S. Wilson, F. Shidlovskiy, B. Buigues, P.G.P. Ericson, M. Germonpré, A. Götherström, P. Iacumin, V. Nikolaev, N. Nowak-Kemp, E. Willerslev, J.R. Knight, G.P. Irzyk, C.S. Perbost, K.M. Fredrikson, T.T. Hardins, S. Sheridan, W. Miller, S.C. Schuster- 2007-Whole-genome shotgun sequencing of mitochondria from ancient hair shafts- Science 317: (#5846, 9/28) 1927-1930
>
> Marande W., G. Gurger- 2007-Mitochondrial DNA as a genomic jigsaw puzzle- Science 318: (#5849, 10/19) 415
>
> Thorsness P.E., T.D. Fox- 1990-Escape of DNA from mitochondria to the nucleus in *Saccharomyces cerevisiae*- Nature 346: 376-379

Most of the proteins and RNAs that operate in the mitochondria have the genes that code for them in the nucleus. These molecules then have to be imported into the mitochondria. Here are studies of this process of **mitochondrial importation** of items:

Braun H-P., U.K. Schmitz- 1999-The protein-import apparatus of plant mitochondria- Planta 209: 267-274

Cheng M.Y., F-U. Hartl, J. Martin, R.A. Pollock, F. Kalousek, W. Neuport, E.M. Hallberg, R.L. Hallberg, A.L. Horwich- 1989-Mitochondrial heat-shock protein hsp60 is essential for assembly of proteins imported into yeast mitochondria- Nature 337: 620-625

Horst M., W. Oppliger, B. Feifel, G. Schatz, B.S. Glick- 1996-The mitochondrial protein import motor: Dissociation of mitochondrial hsp70 from its membrane anchor requires ATP binding rather than ATP hydrolysis- Protein Science 5: 759-767

Kolata G- 1985-How do proteins find mitochondria? They have signaling sequences, investigators find, that tell them where to go- Science 228: 1517-1518

Mahata B., S. Mukherjee, S. Mishra, A. Bandyopadhyay, S. Adhya- 2006-Functional delivery of a cytosolic tRNA into mutant mitochondria of human cells- Science 314: (10/20) 471-474

Schneider H., T. Söllner, K. Dietmeier, C. Edkerskorn, F. Lottspeich, B. Trülasch, W. Neuport, N. Pfanner- 1991-Targeting of the master receptor MOM19 to mitochondria- Science 254: 1659-1662

Being small the **mitochondrial genome** can be altered, so manipulation of this part of the genome is now being examined.

Xu H., S.Z. DeLuca, P.H. O'Farrell- 2008-Manipulating the metazoan mitochondrial genome with targeted restriction enzymes- Science 321: (#5888, 7/25) 575-577

Mitochondria divide in a pattern like **binary fission**. Here is a study of a protein that plays a role in this process.

Lackner L.L., J.S. Horner, J. Nunnari- 2009-Mechanistic analysis of a dynamin effector- Science 325: (#5942, 8/14) 874-877

BIO 107   2010
Day 4, Lecture 9, Title: Respiration II
Text Readings: Campbell et al. (2008) chapter 9.

Topics to cover:

Where we left our story...
Mitochondrial Structure
Oxidative Phosphorylation
    Electron Transport Chain
    Chemiosmosis
Summary

Where we left our story...   Summary: Respiration of glucose so far.
    Fig. 9.6, Respiration overview
        per 1 Glucose in
            In cytosol some things we made (net):
                2 NADH and 2 ATP
            As enter and in matrix some things we made (net):
                6 CO_2, 2 $FADH_2$, 2 ATP, 8 NADH
        Note about substrates used so far:
            ATP is both a product and a substrate.
            Notice that no O_2 has been used.
        When do $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$ then ΔG = -686 Kcal/mole

    Problems yet to deal with....
        accumulating lots of reduced stuff: $FADH_2$ and NADH
            need to find a final home for all the Hs that were taken from the glucose
        recall how fermentation dealt with this material..
        Fig. 9.19, pyruvate use
            either into mitochondria, or use out in cytosol
        Fig. 9.18a, fermentation
            regenerates oxidized form of NAD for use,
                 Does this by reducing the pyruvate, so H put back on to it.
            allows glycolysis to continue, and so makes more ATP
            But since H ends back up in OM, we do not get to much energy
        By doing oxidative respiration includes O_2 as a hydrogen atom acceptor.
            this increases the free energy change greatly.

Mitochondrial Structure

Fig. 6.17, mitochondrial structures

Note:　　　　Outer membrane: has permeable pores that let lots of small molecules pass

Inner membrane: folded into cristae. Site of some important processes.

Intermembrane space: Between above two membranes.

Where many H^+ will be put.

matrix: remnant of original bacterial endosymbiont's cytoplasm

Has bacterial-like DNA, ribosomes, and enzymes of TCA cycle.

MBOTC, quicktime movie, 14.5 mitochondrial shape

Using electron microscopy can reconstruct the spaces and shapes.

Where is Citric Acid cycle located? Where is the pyruvate dehydrogenase?

Oxidative phosphorylation is made up of two parts

Electron Transport Chain and Chemiosmosis

both are transmembrane processes, and involve new forms of energy transduction.

both occur across the inner mitochondrial membrane, and use

matrix space and intermembrane space.

Electron Transport Chain

Fiv. 9.4, NAD redox states

Lots of NADH now made. Need to remove H^+s and their associated electrons.

oxidation of NADH reforms NAD^+, that allows it to get more Hs

ΔG of NADH formation is about +54 Kcal/mole

Fig. 9.16, Chemiosmosis and electron transport chain

Begins with NADH, uses three major membrane spanning complexes

1) NADH oxidase/ubiquinone reductase complex (Complex I)

couples redox reaction with H^+ movement, so it acts as a H^+ translocase

H^+ from matrix moved to intermembrane space

Charge separation, electron one way, H^+ another, makes an electrical potential

across the membrane. Energy transformation.

2) ubiquinone oxidase/cytochrome c reductase complex   (Complex III)

again couples redox Rx with H^+ movement, so it also acts as a H^+ translocase

3) cytochrome c oxidase/oxygen reductase and an H^+ translocase (Complex IV)

this is where oxygen gas is consumed, and the only place

with H^+ movement and water formation,　　　　So ends with water

net for electron transport chain:

NADH becomes NAD^+, so can be used again in to accept Hs from C.

oxidation of NADH has very negative ΔG, about -54 Kcal/mole

So this chemical energy change can power endergonic reactions.

three places where H^+ are moved to intermembrane space,

a concentration gradient is created, requiring energy to create;

also forms membrane potential gradient, an electrical field,

endergonic

energy in redox reaction used to create energy in electrochemical gradient of H^+

$2e^{-1} + 2H^+ + 1/2\ O_2$ becomes water

(Where does each of the substrates come from?

In which compartment does the water get made?)

also an exergonic reaction, so pulls pathway forward

a significant part of the -686 Kcal/mole of glucose oxidation

contributes to H^+ pumping across the membrane.

Note: This is where O_2 is used in respiration.

So oxygen gas is the ultimate acceptor of H.

Fig. 9.13, energy change with electron transport chain

Note where $FADH_2$ enters, note where cytosolic NADH enters

so they differ in number of H^+s moved,

and so differ in contribution to electrochemical gradient

in cardiac muscle cells move NADH into matrix and get more ATP

Chemiosmosis

Review so far:

Used oxidation of carbons in sugars to power formation of ATP.

and accumulated NADH and $FADH_2$

Used electron transport chain to regenerate NAD^+ and FAD.

That allows glycolysis, pyruvate import, and TCA cycle to continue.

Used energy of NADH oxidation to power H^+ pumping across inner mito. membrane

So if do not do something with the electrochemical H^+ gradient the system will back up.

Lots of energy in this gradient, so couple it to ATP synthesis

ATP synthesis has $\Delta G = 7.5$ Kcal/mole

Chemiosmosis as a new means of ATP synthesis.

Fig. 9.16, Chemiosmosis and electron transport chain

Energy in electrochemical H^+ gradient (proton motive force) is used,

and Pi is from solution, not from OM, so NOT substrate level phosphorylation

Peter Mitchell and conceptual problems

Previously only substrate level phosphorylation known

no one anticipated any other mechanism of ATP synthesis.

Previous attempts to isolate mitochondria often disrupted the pmf.

Fig. 9.14, F_iF_o-ATPase, also called ATP synthase

Note membrane spanning subunit, and catalytic subunits.

MBOTC, quicktime movie, 14.1 ATP synthase

Notice that the energy is stored in the ΔpH and ΔE_m

So having intermembrane space is very useful.

Forms ATP in matrix, takes about $3H^+$ moved per ATP made

get roughly 3 ATP/matrix NADH

roughly 2 ATP/$FADH_2$ or cytosolic NADH

These ratios vary as the proton motive force can be used for other things...

What type(s) of energy are being transformed by the ATP synthase?

Summary

Fig. 9.17, summary of oxidative respiration

Note that C-C and C-H bonds are converted to C-O bonds, Carbon_redox.jpg

This acesses energy, so the above are exergonic conversions.

Via substrate phosphorylation powers ATP fromation, which is endergonic

That drives the reduction, of FAD and NAD^+ to $FADH_2$ and NADH

this takes the Hs from the carbons, but are endergonic reactions

Oxidation of $FADH_2$ and NADH is exergonic.

used to power formation of an electrochemical H^+ gradient,

an endergonic reaction since pushes H^+ up concentration and electrical gradient

Also water formation uses oxygen gas as final H acceptor, a very exergonic reaction

this also power the proton motive force across the membrane

ATP synthase allows H^+ to pass down its energy gradient as cross the membrane,

this is exergonic.

This exergonic H^+ flow is coupled through this enzyme to formation of ATP for use.

This is the chemiosmotic mechanism of ATP formation

ATP synthesis takes about 7-10 Kcal/mole, is endergonic.

We use all the ATP in our cells 100s of times per day.

So the ATP in our cells is constantly turning over.

Two fundamental mechanisms of ATP synthesis are used.

Fig. 9.6, substrate-level versus oxidative phosphorylation (i.e. chemiosmosis)

Obviously the later makes most of our ATP.

But the first is the more ancient form of ATP synthesis.

So one process evolved before the other in the history of life.

What are the relative magnitude of energy changes of

Full oxidation of glucose with oxygen to carbon dioxide and water

compared to, ATP and water synthesis from ADP and Pi

compared to, Reduction of NAD^+ with 2H to NADH and H+

What happens if we make the inner membrane permeable to protons?

Pathogens often do this to our cells by secreting items (protonophores).

What happens if oxygen gas is not present?

As when our muscles go anaerobic? Or when bacteria in an infected area use it all up?

(If time, review TCA cycle again...?)

Objectives:

What is chemiosmosis, and what eukaryotic cellular compartment(s) does it involve? What does it create in mitochondria, and what must be present in order for it to take place? What are the substrates and products of chemiosmosis and what enzyme does it use? What is the electron transport chain, and what cellular compartment(s) does it use? Be able to describe the substrates and products of each of the electron transport chain's three major membrane spanning complexes. In the course of oxidative respiration what forms of energy does the cell transduce from beginning to end of all of oxidative respiration?

More ATP are formed with the oxidation of NADH found in the mitochondrial matrix than are formed with the oxidation of $FADH_2$ found there, and also it is more than are formed by the oxidation of cytosolic NADH. Why is this? For each NADH oxidized in the matrix where are protons moved to, and why does their destination matter?

Where in the process of oxidative respiration is oxygen gas consumed?

Know the parts of mitochondria, and the areas of the cell in which each of the major stages of oxidative respiration occurs. Be able to relate the parts of the mitochondria that are derived from corresponding parts of a free living bacterium.

If a compound was added to the mitochondria that could accept electrons from reduced cytochrome c so that it did not pass any electrons further down the electron transport chain what effect would this have on: The proton concentration gradient across the inner mitochondrial membrane? The consumption of oxygen? The rate of glycolysis? The regeneration of NAD^+ in the mitochondrial matrix? The rate of carbon dioxide production? The rate of ATP production?

For one glucose respired in the presence of oxygen what would be the number of ATPs that would be expected to be produced? What if you started with just one pyruvate, or one NADH found in the mitochondrial matrix? What amount of ATP would be expected to be made if the cell lacked oxygen?

For review, see self-quiz questions #2, 4, 5 and 6 of chapter 9.

Needed overheads and items:

Fig. 9.6, Respiration overview
Fig. 9.19, pyruvate use
Fig. 9.18a, fermentation
Fig. 6.17, mitochondrial structures
MBOTC, quicktime movie, 14.5 mitochondrial shape
Fig. 9.4, NADH redox states
Fig. 9.16, Chemiosmosis and electron transport chain
Fig. 9.13, free energy change with electron transport
Fig. 9.16, Chemiosmosis and electron transport chain
Fig. 9.14, F_iF_o-ATPase
MBOTC, quicktime movie, 14.1 ATP synthase
Fig. 9.17, summary of oxidative respiration
carbon_redox.jpg (Weber, 2000)
Fig. 9.6, substrate-level versus oxidative phosphorylation

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002-<u>Molecular biology of the cell. Cell Biology Interactive CD</u>. Movies 14.1 and 14.5. Garland Science Press. N.Y., N.Y.

Baum H- *Waltz round the Cycle*- from <u>The Biochemists' Songbook</u>. Metabolic Melodies, London, U.K.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Cellular respiration. Harvesting chemical energy. Chapter 9. Pages 162-184. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Cram J.M., D.J. Cram- 1978-<u>The Essence of Organic Chemistry</u>. Pg. 35. Addison-Wesley Publishing Co., Reading, MA.

Weber A.L- 2000-Sugars as the optimal biosynthetic carbon substrate of aqueous life throughout the universe- Origins of Life and Evolution of the Biosphere 30: 33-43

Related issues:

Here are studies of the structure and function of the F_1F_0-**ATP synthase** of the mitochondria.

Collinson I.R., I.M. Fearnley, J.M. Skehel, M.J. Rusnwick, J.E. Walker- 1994-ATP synthase from bovine heart mitochondria: Identification by proteolysis of sites in F_0 exposed by removal of F_1, and the oligomycin-sensitivity conferral protein- Biochemistry Journal 303: 639-645

Furuike S., M.D. Hossain, Y. Maki, K. Adachi, T. Suzuki, A. Kohori, H. Itoh, M. Yoshida, K. Kinosita jr.- 2008-Axle-less F_1-ATPase rotates in the correct direction- Science 319: (#5865, 2/15) 955-958

Lippe G., F.D. Sala, M.C. Sorgato- 1988-ATP synthase complex from beef heart mitochondria: Role of the thiol group of the 25-KDa subunit of F_0 in the coupling mechanism between F_0 and F_1- Journal of Biological Chemistry 263: (#35) 18627-18634

Lutter R., M. Saraste, H.S. vanWalraven, M.J. Runswick, M. Finel, J.F. Deatherage, J.E. Walker- 1993-F_1F_0-ATP synthase from bovine heart mitochondria: Development of the purification of a monodisperse oligomycin-sensitive ATPase- Biochemistry Journal 295: 799-806

Ouazzani C., A. Berville- 1991-Isolation and antigenic characterization of sugar beet (*Beta vulgaris* L.) mitochondria F_1-ATPase: Studies of some *Beta* species and of the cytoplasmic male sterile Owen form- Plant Science 74: 53-64

Mitochondria play a role in **programmed cell death** (i.e. **apoptosis**), which is one way our immune system destroys infected cells:

Chang W-H., C-H. Chen, R-J. Gau, C-C. Lin, C-L. Tsai, K. Tsai, K-J. Lu- 2002-Effect of baicalein on apoptosis of the human Hep G2 cell line was induced by mitochondrial dysfunction- Planta Medica 68: (#4) 302-306

France-Lanord V., B Brugg, P.P. Michel, Y. Agid, M. Ruberg- 1997-Mitochondrial free radicals signal in ceramide-dependent apoptosis: A putative mechanism for neuronal death in Parkinson's disease- Journal of Neurochemistry 69: 1612-1621

Kluck R.M., E. Bossy-Wetzel, D.R. Green, D.D. Newmeyer- 1997-The release of cytochrome C from mitochondria: A primary site for Bcl-2 regulation of apoptosis- Science 275: 1132-1136

Quillet-Mary A., J-P. Jaffrézou, V. Mansat, C. Bordier, J. Naval, G. Laurent- 1997-Implication of mitochondrial hydrogen peroxide generation in ceramide-induced apoptosis- Journal of Biological Chemistry 272: (34) 21388-21395

There have been reports that **aging** alters the activities in the mitochondria and that changes in these activities might be related to a species' **life span**:

Hajnóczky G., J.B. Hoek- 2007-Mitochondrial longevity pathways- Science 315: (2/2) 607-609

Hayakawa M., S. Sugiyamma, K. Hattori, M. Takasawa, T. Ozawa- 1993-Age-associated damage in mitochondrial DNA in human hearts- Molecular and Cellular Biochemistry 119: 95-103

Mecocci P., U. MacGarvey, A.E. Kaufman, D. Koontz, J.M. Shoffner, D.C. Wallace, M.F. Beal- 1993-Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain- Annals of Neurology 34: (#4) 609-616

Nagley P., I.R. MacKay, A. Baumer, R.J. Maxwell, F. Vaillant, Z-X. Wang, C. Zhang, A.W. Linnane- 1992-Mitochondrial DNA mutation associated with aging and degenerative disease- Annals of the New York Academy of Science 673: 92-102

Pinton P., A. Rimessi, S. Marchi, F. Orsini, E. Migliaccio, M. Giorgio, C. Contursi, S. Minucci, F. Mantovani, M.R. Wieckowski, G.D. Sal, P.G. Pelicci, R. Rizzuto- 2007-Protein kinase Cβ and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66[Shc]- Science 315: (2/2) 659-663

Richter C- 1995-Oxidative damage to mitochondrial DNA and its relationship to ageing- International Journal of Biochemistry and Cell Biology 27: (#7) 647-653

Shigenaga M.K., T.M. Hagen, B.N. Ames- 1994-Oxidative damage and mitochondrial decay in aging- Proceedings of the National Academy of Science (USA) 91: 10771-10778

Wallace D.C- 1997-Mitochondrial DNA in aging and disease- Scientific American 277: (#2) 40-47

Wallace D.C., J.M. Shoffner, I. Trounce, M.D. Brown, S.W. Ballinger, M. Corral-Debrinski, T. Horton, A.S. Jun, M.T. Lott- 1995-Mitochondrial DNA mutations in human degenerative diseases and aging- Biochemica et Biophysica Acta 1271: 141-151

Here is the citation of the original article by Peter Mitchell in which he lays out the thinking for the **chemiosmotic hypothesis**. (Unfortunately the article is peppered with misleading references to "high energy bonds"!)

Mitchell P- 1961-Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism- Nature 191: (#4784, 7/8) 144-148

Other studies of the **proton-motive force** across the inner mitochondrial membrane:

Berry E.A., P.C. Hinkle- 1983-Measurement of the electrochemical proton gradient in submitochondrial particles- Journal of Biological Chemistry 258: (#3) 1474-1486
Brand M.D., P. Couture, P.L. Else, K.W. Withers, A.J. Hulbert- 1991-Evolution of energy metabolism. Proton permeability of the inner membrane of liver mitochondria is greater in mammal than in a reptile- Biochemistry Journal 275: 81-86

This article has a summary of the steps in **cellular respiration** and sums the **ATP production** of the various stages and how the ATP is typically used.

Brand M- 2003-Approximate yield of ATP from glucose, designed by Donald Nicholson- Biochemistry and Molecular Biology Education 31: (#1) 2-4

BIO 107   2010
Day 4, Lecture 10, Title: Photosynthesis
Text Readings: Campbell et al. (2008), Chapter 10.

Topics to cover:

> **Respiration vs. Photosynthesis**
> **Chloroplast structure**
> **Light Reactions**
> **Dark Reactions**
> > **Rubisco and Photorespiration**
> **C$_4$ and CAM Options**

Respiration vs. Photosynthesis
>   Fig. 10.16, Chloroplast vs. mitochondria, chemiosmosis
>   Superficially the H$^+$ looks like being moved in opposite directions, but really same
> >   cytosolic-like compartment has ATP formation,
> >   extracellular-like space has acidic space.
> >   So orientation of H$^+$ movement and location of ATP formation is the same.
>   Review endosymbiosis and how the orientations got this way.
>   On black board draw mitochondria with cristae in eukaryotic cell.
> >   Carbon chemistry in cytosol, C6 to C3 (Glycolysis)
> >   Carbon chemistry in matrix
> > >   C3 to carbon dioxide (C1) (Pyruvate import and Krebs cycle)
> > >   Electron transport system, three protein complexes
> > > >   proton motive force generation
> > >   Chemiosmosis and ATP formation
>   Then on board draw chloroplast for photosynthesis and review
> >   Make C3 in chloroplast from C1, need free energy to drive this
> > >   need reducing power (H) to reduce the carbons
> >   Use chemiosmosis to make ATP, but need pmf to drive this
> >   Need electron transport system to create pmf
> > >   has several major membrane complexes, need to move electrons to do this
> >   Take electrons from water, but need energy to do this
> >   Absorb light to power

Chloroplast structure
>   Fig. 10.3, leaf, chloroplast structures
>   Three membranes: Inner and outer membranes, thylakoids
>   Three soluble spaces: Stroma, thylakoid space, intermembrane space
>   How CO$_2$ enters relative to leaf organ, note structures
> >   stomata, leaf air space, cell, chloroplast
> >   this leads to water loss, and leaf cells are endangered!
> >   So leaves have stomates to regulate CO$_2$ entry and water loss.
> > >   Surface of leaves have a waxy cuticle to regulate water loss.

Vascular tissue (xylem) moves water to leaves from roots.
Most water taken to leaves is lost out of the leaves so that CO_2 can enter.

Light Reactions
Fig. 10.5, overview of light reactions
Two parts to typical photosynthesis: light reactions, dark reactions
Goal is to drive carbon fixation/reduction to make more organic matter.
Light reactions
Fig. 10.5, layer 2, overview of light reactions
Start with:
light, ADP + P_i, water, $NADP^+ \rightarrow O_2$, NADPH, ATP and water
analogous to electron transport chain in mitochondria
note ATP and NADPH are put in Stroma = cytosolic-like space
thylakoid membranes provide means for charge separation, and chemiosmosis
Fig. 10.17, light reactions
Go through steps
Light used for charge separation
water splitting, H^+ pumping, electron flow to $NADP^+$
electrochemical gradient powers chemiosmotic ATP synthase
note the H^+ from water splitting is left in the thylakoid lumen
P.S. II, reaction center optimal light abs. at 680 nm
Water oxidase/plastoquinone reductase
Cytochrome complex
plastoquinone oxidase/plastocyanin reductase
and moves H^+ to create proton motive force (pmf)
P.S. I, reaction center optimal light abs. at 700 nm
plastocyanin oxidase/Ferredoxin reductase
ultimately $NADP^+$ reduced to NADPH
So e^{-1} from water, and H^+ picked up from solution.
This gives us H to be delivered later to carbons to reduce them.
Endergonic steps include: Water splitting, NADPH formation, and
creation of H^+ electrochemical gradient
So need light energy to drive the above endergonic reactions.
NADP structure
Racusen (1977) file: NADH.jpg
just like NAD but with a phosphate group
Generally NAD used to oxidize C
NADPH used to donate H to reduce C
Oxidation of NADPH + H^+ to $NADP^+$ + 2H
has free energy change of about -55 Kcal/mole

Chlorophyll structure. A light absorbing pigment, other pigments exist as well...

    Fig. 10.10, chlorophyll

    Heme, Racisen (1977) file: heme.jpg

        Chlorophylls and other pigments occur in complexes, transfer energy

        Note how chlorophyll is similar to heme group in hemoglobin

        Implies a similar biosynthetic pathway, and so evolutionary relatedness?

Light harvesting complex has many types of pigments arranged around a reaction center

    Fig. 10.12, Light harvesting complex and reaction center

    note locations of light absorbing chlorophylls versus reactive chlorophyll

    couples photon energy to redox reactions and electrochemical gradient formation

Dark Reactions

    Fig. 10.5, layer 3, overview with dark reactions

    The purpose is to "fix" the carbon into organic matter.

        Carbon "fixation" involves no direct reduction, just gets C into organic matter

        later uses Hs to reduce Cs, but this is not the RUBISCO reaction

        So reducing CO_2 to level of carbohydrates (CH_2O). Very endergonic.

    Use the products of light reactions to drive endogonic reactions and as reducing power

        ATP hydrolysis and NADPH oxidation are very exergonic reactions.

    Fig. 10.18, Calvin cycle

    Note: Glucose is NOT a product of the Calvin cycle...

General reaction of P.S. depends on definition... for dark reactions:

    $3 CO_2 + 9 ATP + 9 H_2O + 6 NADPH =$

        Glyceraldehyde-3-phosphate (G3P) $+ 9 ADP + 9 P_i + 6 NADP^+$
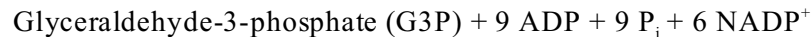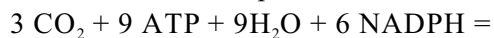
    In stroma makes a triose-phosphate (G3P), not glucose,

        may convert this sugar to starch in the chloroplast, forming a starch grain

    No O_2 directly used or made in the dark reactions, that was a light reaction product.

    Light reactions then regenerate ATP and NADPH

Fig. 10.21, Review of photosynthesis

    Review light and dark reactions

    Note G3P can go to starch in stroma, or be exported to cytosol and converted to sucrose

    most plants send sucrose from cell to cell, not glucose

    So the product of photosynthesis that is exported from the chloroplast is a triose-P sugar.

        The product of photosynthesis that the cell typically exports is sucrose.

        Glucose just is not accumulated, though it is made in the cell to make the sucrose.

Rubisco and Photorespiration

    RUBISCO, a major enzyme for carbon fixation used in the Calvin cycle

        This name stands for: ribulose bisphosphate carboxygenase/oxygenase

        It is slow! Has about 1 turnover per second!

            Some enzymes turn over a million times per second.

        To make up for its slow catalysis it must be at very high concentration.

            So exists at mM concentration in stroma, unheard of for an enzyme.

Reaction it catalyzes: (RUBP.tif)
    $RuBP + CO_2 =$ two 3-phosphoglycerate
    C5 + C1 = 2 C3
    3-Phosphoglycerate (3-PGA) is an acid, not a sugar.
    Note that this reaction uses no ATP, and no NADPH.
    Need later to get Hs to reduce the carbon to the level of sugars.
Photorespiration is a problem seen with RUBISCO.
    RUBISCO has affinity for CO_2 and for O_2, they both compete for binding site
        So CO_2 and O_2 are both substrates for this enzyme.
            O_2 is an alternate substrate, NOT an inhibitor.
        Obviously using O_2 oxidizes the carbons, and is not desirable. (RUBP2.tif)
            $C5 + O_2 = C3 + C2$
    molecular shapes of these two gases are similar
    When carbon fixation began (over 2.5 billion years ago) no free O_2 in the
        atmosphere, so initially there was no need to discriminate?
    Now stuck with it, evidence of an evolutionary remnant
        a good example of an inefficient enzyme.
            This is one of the most common enzymes on the planet!
    25-30% of the time it uses oxygen as a substrate
    pathway recovers some carbon, removes a toxin, but does make some CO_2
    called "photorespiration" as it consumes O_2. and release CO_2.

C_4 and CAM Options
    Fig. 10.19, C4
    C4_leaf_anatomy.jpg (Esau 1977, fig. 19.10)
        A means to move and concentrate a vital gas, in this case CO_2.
            (Contrast with our moving gases through the tissues of our body.)
        shunt carbon dioxide into cells with rubisco enzyme
        C3 + C1 = C4, then after move it, C4 = C3 + C1
        raise concentration of carbon dioxide above Km, so little oxygen used by rubisco
        What does this do to concentration of carbon dioxide in leaf air space?
        What about concentration gradient across stomatal pore?
        Do stoma have to be open more or less?
        What does this do to water loss and ability to tolerate hot/dry conditions?
        Corn and sugar cane can live in tropics

    Fig. 10.20, C4 and CAM
        CAM system:
        Do one step at night, other at day
        so stomata can be kept closed during day, and limit water loss
        done by desert plants

These are modifications to conserve water, and so see C4 and CAM in water stressed areas.

Objectives:

  In what ways are the photosynthetic processes done in the chloroplast similar to respiratory processes done in the mitochondria? Be able to compare the compartments, pathways, and redox states of carbon of these two processes. Be able to compare how chemiosmosis is used in photosynthesis and respiration.

  Be able to describe for the light and dark reactions of photosynthesis: The substrates and products of each and compartments of the chloroplast involved. What role does water, NADPH, ATP and the thylakoid membranes play in photosynthesis? In carrying out photosynthesis what forms of energy are transduced? In the light reactions, and later in the dark reactions, what exergonic reactions are used to power endergonic steps? What type of carbon compound does the chloroplast normally export to the rest of the cell as a result of photosynthesis? What compound does a cell in a leaf normally export to the rest of the plant?

  RUBISCO is a major enzyme in the Calvin cycle. What reaction does it catalyze and what are all its possible substrates? How does photorespiration complicate the fixation of carbon into organic matter by the plant? Be able to determine whether either of these reactions (carbon fixation and photorespiration) are redox reactions.

  During photosynthesis what is happening to the redox state of carbon? What is needed for these changes to be accomplished? In the chloroplast what is the ultimate source of the electrons and hydrogen ions that are used to reduce the carbons?

  Describe the path that carbon dioxide typically takes through a leaf as it goes from outside a plant up to its fixation in a chloroplast. Be able to describe the carbon dioxide concentrating mechanisms used by C4 and CAM plants. How does each modify the activity of their dark reactions in terms of space and time? What does this mechanism do to the plant's ability to restrict water loss?

For chapter 10 see self-quiz questions #1, 3, 4, 5 and 7 for review.

Needed overheads and items:

Fig. 10.16, Chloroplast vs. mitochondria, chemiosmosis
Fig. 10.3, leaf, chloroplast structures
Fig. 10.5, overview of light reactions
Fig. 10.5, layer 2, overview of light reactions
Fig. 10.17, light reactions
NADP, Racusen (1977) file: NADH.jpg
Fig. 10.10, chlorophyll
Heme, Racusen (1977) file: heme.jpg
Fig. 10.12, Photosynthetic reaction center and light harvesting pigments
Fig. 10.5, layer 3, overview with dark reactions
Fig. 10.18, Calvin cycle
Fig. 10.21, Review of photosynthesis
RUBP.tif
RUBP2.tif
Fig. 10.19, C4
C4_leaf_anatomy.jpg (Esau 1977, fig. 19.10)
Fig. 10.20, C4 and CAM

Handout: Rubisco reactions, RUBP.tif, RUBP2.tif

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Photosynthesis. Chapter 10. Pages 185-205. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Esau K- 1977-<u>Anatomy of Seed Plants</u>. 2nd edition. Fig. 19.10. John Wiley and Sons. N.Y., N.Y.

Racusen D- 1977-<u>A pocket guide to bio-organic structures</u>- University of Vermont Press. Camden, Maine.

Related issues:

The Calvin cycle is actually just one pathway used by autotrophs. There are five types of **carbon fixation pathways** known. There are also various types of RUBISCO enzymes:

Sato T., H. Atomi, T. Imanaka- 2007-Archaeal type III RUBISCO, function in a pathway for AMP metabolism- Science 315: (2/16) 1003-1006

Thauer R.K- 2007-A fifth pathway for carbon fixation- Science 318: (#5857, 12/14) 1732-1733

White D- 2000-Photosynthesis- Chapter 5, pgs. 132-156, in, The Physiology and Biochemistry of Prokaryotes. 2nd edition. Oxford University Press, N.Y.

White A- 2000-C_1 metabolism- Chapter 13, pgs. 338-362, in, The Physiology and Biochemistry of Prokaryotes. 2nd edition. Oxford University Press, N.Y.

Chloroplasts have their own DNA and genes. The **chloroplast genome** must coordinate with the nuclear genome in the same cell to operate.

Koussevitzky S., A. Nott, T.C. Mockler, F. Hong, G. Sachetto-Martins, M. Surpin, J. Lim, R. Mittler, J. Chory- 2007-Signals from chloroplasts converge to regulate nuclear gene expression- Science 316: (5/4) 715-719

Timme R.E., J.V. Kuehl, J.L. Boore, R.K. Jansen- 2007-A comparative analysis of the *Lactuca* and *Helianthus* (Asteraceae) plastid genomes: Identication of divergent regions and categorization of shared repeats- American Journal of Botany 94: (#3) 302-312

Zhang D-P- 2007-Signaling to the nucleus with a loaded gun- Science 316: (5/4) 700-701

Since most of the proteins in the chloroplast are coded for by genes in the nucleus, there has to be a means of **protein importation**:

Lübeck J., L. Heins, J. Soll- 1997-Protein import into chloroplasts- Physiologica Plantarum 100: 53-64

There are five lineages in the bacteria that are known to do photosynthesis. Here is an article about a member of one of these **photosynthetic bacterial** lineages:

Bryant D.A., A.M. Garcia-Costas, J.A. Maresca, A.G.M. Chew, C.G. Klatt, M.M. Batescon, L.J. Tallon, J. Hostetler, W.C. Nelson, J.F. Heidelberg, D.M. Ward- 2007-*Candidatus* chloracidobacterium thermophilium: An aerobic phototrophic acidobacterium- Science 317: (#5837, 7/27) 523-526

The structure of the **photosynthetic membrane complexes** is of great interest. In recent years several of them have had their structure examined in terms of how they function:

> Lee H., Y-C. Cheng, G.R. Fleming- 2007-Coherence dynamics in photosynthesis: Protein protection of excitonic coherence- Science 316: (6/8) 1462-1465
>
> Skourtis S.S., D.N. Beratan- 2007-Photosynthesis from the protein's perspective- Science 316: (5/4) 703-704
>
> Wang H., S. Liu, J.P. Allen, J.C. Williams, S. Blankert, C. Laser, N.W. Woodbury- 2007-Protein dynamics control the kinetics of initial electron transfer in photosynthesis- Science 316: (5/4) 747-750
>
> Yachandra V.K., V.J. DeRose, M.J. Latimer, I. Mukerji, K. Sauer, M.P. Klein- 1993- Where plants make oxygen: A structural model for the photosynthetic oxygen-evolving manganese complex- Science 260: 675-678

Origins of photosynthesis. With the discovery of light absorption by deep sea microbes, some propose that photosynthesis may have evolved from a similar system billions of years ago:

> Bohannon J- 2005-Microbe may push photosynthesis into deep water- Science 308: (6/24) 1855

The most **common photosynthetic organisms**, and those that do most of the photosynthesis on the Earth, are NOT plants. They are bacteria. Here are some articles about photosynthesis in them.

> Nadis S- 2003-The cells that rule the seas- Scientific American 289: (#6) 52-53
>
> Wöhri A.B., G. Katona, L.C. Johansson, E. Fritz, E. Maimerberg, M. Andersson, J. Vincent, M. Eklund, M. Cammarata, M. Wulff, J. Davidsson, G. Groenhof, R. Neutze- 2010-Light-induced structural changes in a photosynthetic reaction center caught by Laue diffraction- Science 328: (#5978, 4/30) 630-633

Study of the **proton gradient** created across the thylakoid membranes in chloroplasts continue:

> Ewy R.G., R.A. Dilley- 2000-Distinguishing between luminal and localized proton buffering pools in thylakoid membranes- Plant Physiology 122: 583-595

BIO 107   2010
Day 4, Lecture 11, Title: Signal Reception, Transduction, Response
Text Readings: Campbell et al. (2008) Chapter 11.

Topics to cover:

> **Overview**
> **Example: Bacterial Two Component System**
> **Three Classes of Receptors**
> **Transduction options**
> **Responses**
> **Some variations**
> **Implications**

Overview
Signal-transduction systems
> Fig. 11.6 overview of cell signaling
> signals and receptors: This relates to detecting external signals,
> > but could be an internal signal in the cell, or detected in the cell.
> transduction: Getting the information (not the signal) from the receptor through the cell.
> response: Will not deal much with this, but this can be behavioral, growth,
> > gene expression changes, etc...

Example: Bacterial Two Component System
Bacterial two-component system (White, 2000) Not in book...
> This system is common to many pathogenic bacteria, fungi, etc.
> > So are targets for some antibacterial drugs...
> Fig. 18.1 (White, 2000) File: two_component.jpg
> Kinases: Add phosphate (P_i) to substrate. Phosphatases: Remove P_i from item.
> Histidine kinase (HK), here it is a membrane spanning protein
> > two binding sites: signal binding site, and kinase active site
> > > first phosphorylates its own HIS with P_i from ATP
> > > then donates the phosphate to RR
> Response regulator (RR), a protein
> > inactive when not phosphorylated
> > active when phosphorylated, and can move through rest of cell
> > > it's binding site is now available to bind to and alter other activities...
> Phosphatase
> > resets RR to inactive state
> bacteria may use this to switch from aerobic to anaerobic
> > respiration, or turn on genes for sugar metabolism

Three Classes of Receptors
There are many others, we will focus on three major classes of receptors.
Receptors in membranes are common, all three of these classes are membrane spanning proteins.
Fig. 11.7, Transmembrane receptor
Review structure of a membrane-spanning protein, note:
amine end, carboxyl end, conserved domains,
Can duplicate this system, then vary, get different systems with different binding sites.
So once have a receptor evolution allows production of others...
All receptors depend on changes in protein shape and activity with signal reception.

G protein coupled receptors (GPCR)
Fig. 11.7, G-protein system
The receptor spans the membrane.
On cytosolic side there is an associated G protein.
G proteins, bind GTP (like ATP but with G rather than A nitrogenous base)
GTP binding activates the protein allows it to bind other proteins
gets GTP when the associated receptor is activated
G proteins have a slow GTPase activity that will turn system off
note need to turn system off if want to use it again
So change in protein shape alters activity of other proteins
Tyrosine-kinase system
Fig. 11.7, tyrosine-kinase system
This receptor has an extracellular binding site, two subunits combine on signal binding
binding there activates TYR-kinase activity on cytosolic side.
First, it phosphorylates itself.
Then can phosphorylate other proteins, need a phosphatase to remove the P_is and reset
So reception can lead to covalent modification of other items.
(Notice how this is similar to the bacterial 2-component system...?)
Ligand-gated ion channel system
Fig. 11.7, ligand-gated ion channel system
reception of signal (ligand) binds at extracellular binding site.
This binding opens channel, and let ions pass through.
change in [ion] alters enzyme activity
need to pump ions out to reset system
Note analogy to what happens at human synapse
How is this like allosteric regulation, how is it different from what we saw before?
So binding alters channel shape and activity.

In all three of these cases:
Binding at a specific binding site leads to changes in protein structure.
That alters some other item, but inside the cell.
So receptors get the INFORMATION of the signal across the cell membrane.
?Which of these three would likely work the fastest?

2

Transduction options

So information of signal reception is in cell, what next?

Depends on whether need a fast or a slow connection to a response.

If fast then may use amplification as a part of transduction,

note the "signal" is not amplified just information

(The text often describes "amplification of the signal" but this is not what happens.)

amplification costs lots of energy! Can be done in several ways...

Altering proteins: Phosphorylation cascades increase number of activated proteins

Fig. 11.15, transduction amplification with cascade

one signal molecule binding can result in millions of activated molecules in cell

Fig. 11.9, phosphorylation cascade

note need for phosphatases to turn it off, costs lots of ATPs

Some non-protein items used by changing their concentration in the cytosol.

These items are called secondary messengers.

cAMP, a secondary messenger

Fig. 11.11, secondary messenger,

definition; change in concentration in a compartment of the cell

In this case the enzyme adenylyl cyclase makes cAMP.

then cAMP binds at binding sites in the cell.

Fig. 11.10, cAMP

Need phosphodiesterase to destroy the cAMP and reset system.

So transient change in concentration in a cell compartment as transduction step

Calcium ions, another secondary messenger

Fig. 11.12, Ca^{+2} pumps in cells

note error for mitochondrial intermembrane space in this figure

we do not store Ca^{+2} in the mitochondrial matrix.

Reasons for keeping [calcium ion] low in cytosol and mitochondrial matrix

calcium phosphate can be made if Ca^{+2} and PO_4^{-2} are together

want nM to μM concentration in cytosol and matrix

if it gets higher than that can get crystals forming in our cell!

Fig. 11.13, Ca^{+2} and IP_3 system, also secondary messengers

note binding of Ca^{+2} by calmodulin

so rise in secondary messenger concentration is detected

use pumps to recover by removing Ca^{+2} from the cytosol

Inositol-triphosphate (IP_3) and Diacylglycerol (DAG), other secondary messengers

activate and enzyme to degrade phospholipid in the plasma membrane

this can release inositol-triphosphate (IP_3) into cytosol

also leaves Diacylglycerol in membrane (DAG)

So one is a soluble and one a hydrophobic secondary messenger
membrane proteins can have hydrophobic binding sites
Which has larger volume membrane or cytosol?
So which would be filled with secondary messenger faster?

Receptor binding changes shape of perhaps one receptor molecule on its cytosolic side.
If that activates just one protein in the cell then it could take hours/days/weeks
for that protein to have an effect at a spot in the cell far away...
That is OK if signal to response connection can be slow.
Use the above transduction amplification options to make the connection faster.
See this in pathogen detection, hormonal responses, sensory cells, etc....
Faster responses cost the cell energy, so can save energy with fewer transduction steps...

Responses
Immediate changes in enzyme activity and protein binding, ex: muscle contraction
Long term changes in gene expression
Fig. 11.14, altering gene expression
via DNA-binding proteins, called transcription factors (TF)
can take hours to days... as few transductive steps and little amplification...

Some variations
Signal cross talk
The examples given so far have been linear, but they can branch and cross connect
Fig. 11.17, signal system cross talk
This allows many inputs, to be summed. One input to have multiple effects...
Or for an output to depend on the presence of multiple independent inputs...
Fig. 11.18, scaffolding proteins
When activated can hold other proteins in right configuration.
So multiple protein complexes can be important.

Signals with internal receptors
The receptors described earlier were membrane spanning.
Some receptors can be in side of the cell.
hydrophobic signals such as steroids can cross the membrane easily and get into the cell
Fig. 11.8, steroid signal and intracellular receptor
so not all receptors are at the membrane
? Which can be removed faster a chemical signal that can or cannot cross our cell
membranes?

Cell-to-cell signaling
    The presence of receptors allows cells to send signals to other cells
    Fig. 11.5, Animal cell-to-cell communication
    Three examples of what we call some of these signals is given in this figure.
            Local growth regulators
            synaptic signals
            hormones in blood
            pheromones?
    Fig. 11.4, symplastic and apoplastic communication
    May send a signal via connected cytoplasms, through gap junctions.
    Or may have receptor bind to surface signal on cell
        so signals do not need to be released from the cell

Implications
    Uses at various levels of cell signaling systems
        Intracellular: coordination of organelle functions
        Cell sensing and responses to environment
        Multicellular organismal intra-organismal
            cell-to-cell coordination of actions
        Interorganismal
            mating
        Interspecial
            ecological implications, symbiosis

    So there is natural selection for function receptor/transduction/response systems.
        New combinations of elements of these systems produce diversity.
        Thus new systems have evolved over time.

Objectives:

What models do we have to explain how information indicating the presence of external signals gets transmitted across the plasma membrane and results in changes in cellular behavior or growth?

What difference does it make if the signal-molecule is hydrophobic or hydrophilic in terms of where the cell may have a receptor for it? Be able to describe what typically happens at the molecular level during signal reception. Be able to describe the three major classes of receptors we covered. Which of these three types of receptors is most likely to be fastest in affect?

Be able to describe the bacterial two-component system as an example of prokaryotic signal reception and transduction, and compare it to the eukaryotic receptors we cover.

What options do cells have for internal signal transduction? Be able to describe examples in which the cell modifies existing proteins as well as how the cell might alter the concentration of secondary messengers in a cellular compartment. What is one consequence of having many transduction steps in series between the reception and the response to a signal in terms of the speed of the system? What must occur for this arrangement to be adaptive and so be selected for through evolutionary processes?

What is one consequence of different signals activating different receptors that then activate transduction using the same secondary messenger in the same cell? Will this always result in a common response to different signals? How can a rise in calcium ion concentration in a muscle cell's cytosol cause one type of response, while a rise in calcium ion concentration in a nerve cell's cytosol causes a different type of response?

After responding to a signal what does the cell have to do to turn off the response and reset the transduction system? Given an example of a signal transduction system (for instance, see figure 11.13) be able to describe what will have to be done to reset the system. What must be done to each of the three receptor classes after use to reset them for subsequent signal reception?

Self quiz questions #2, 3, 6 and 7 of chapter 11 are worth a look.

Needed overheads and items:

       Fig. 11.6, overview of cell signaling
       Fig. 18.1 (White, 2000) File: two_component.jpg
       Fig. 11.7, Transmembrane receptor
       Fig. 11.7, G-protein system
       Fig. 11.7, tyrosine-kinase system
       Fig. 11.7, ligand-gated ion channel system
       Fig. 11.15, amplification with cascade
       Fig. 11.9, phosphorylation cascade
       Fig. 11.11, secondary messenger, cAMP
       Fig. 11.10, cAMP
       Fig. 11.12, Ca ion pumps in cells
       Fig. 11.13, Ca^{+2} and IP_3 system
       Fig. 11.14, altering gene expression
       Fig. 11.17, signal system cross talk
       Fig. 11.18, scaffolding proteins
       Fig. 11.8, steroid signal and intracellular receptor
       Fig. 11.5, Animal cell-to-cell communication
       Fig. 11.4, symplastic and apoplastic communication

Handout: Fig. 18.1 (White, 2000) File: two_component.jpg

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Cell communication. Chapter 11. Pages 206-227. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

White D- 2000-Adaptive and developmental changes- Chapter 18, pgs. 434-533, in, <u>The Physiology and Biochemistry of Prokaryotes</u>. 2nd edition. Oxford University Press, N.Y.

Related issues:

There are other types of **secondary messengers**. Here are articles that point out the roles of cyclicADP, other forms of inositol phosphates (IP), and the uses of heme as a secondary messenger:

Dodd A.N., M.J. Gardner, C.T. Hotta, K.E. Hubbard, N. Dalchau, J. Love, J-M. Assie, F.C. Robertson, M.K. Jakobsen, J. Gonçalves, D. Sanders, A.A.R. Webb- 2007- The *Arabidopsis* circadian clock incorporates a cADPR-based feedback loop- Science 318: (#5858, 12/14) 1789-1792

Huang Y.H., J.A. Grasis, A.T. Miller, R. Xu, S. Soonthornvacharin, A.H. Andreotti, C.D. Tsoukas, M.P. Cooke, K. Sauer- 2007-Positive regulation of Itk PH domain function by soluble IP_4- Science 316: (5/11) 886-889

Illies C., J. Gromada, R. Flume, B. Leibiger, J. Yu, K. Jubs, S-N. Yang, D.K. Barma, J.R. Falck, A. Saiardi, C.J. Barker, P-O. Berggren- 2007-Requirement of inositol pyrophosphates for full exocytotic capacity in pancreatic ß cells- Science 318: (#5854, 11/23) 1299-1302

Irvine R- 2007-The art of the soluble- Science 316: (5/11) 845-846

Lee Y-S., S. Mulugu, J.D. York, G.K. O'Shea- 2007-Regulation of a cyclin-CDK-CDK inhibitor complex by inositol pyrophosphates- Science 316: (4/6) 109-112

Mulugu S., W. Bai, P.C. Fridy, R.J. Bastidas, J.C. Otto, D.E. Dollins, T.A. Haystead, A.A. Ribeiro, J.D. York- 2007-A conserved family of enzymes that phosphorylate inositol hexakisphosphate- Science 316: (4/6) 106-109

Nagamutsu S., M. Ohara-Imaízum- 2007-IP_7 debut in insulin release- Science 318: (#5854, 11/23) 1249-1250

Yin L., N. Wu, J.C. Curtin, M. Qatanani, N.R. Szwergold, R.A. Reid, G.W. Waitt, D.J. Parks, K.H. Pearce, G.B. Wisely, M.A. Lazar- 2007-Rev-erbα, a heme sensor that coordinates metabolic and circadian pathways- Science 318: (#5857, 12/14) 1786-1789

Various types of kinases both **protein kinases** and others have been found. Here are articles that study aspects of them and their role in signal transduction:

Hawkins P.T., L.R. Stephens- 2007-PI3Kγ is a key regulator of inflammatory responses and cardiovascular homeostasis- Science 318: (#5847, 10/5) 64-66

Lee J.Y., J.A. Engelman, L.C. Cantley- PI3K charges ahead- Science 317: (#5835, 7/13) 206-207

Townley R., L. Shapiro- 2007-Crystal structures of the adenylate sensor from fission yeast AMP-activated protein kinase- Science 315: (3/23) 1726-1729

Which proteins are phosphorylated, and how **patterns of phosphoryaltion** differ in different cell types is of great interest, and changes in transduction systems can play a role in speciation. How the **phosphatases** and **kinases** interact to achieve these patterns is also of interes. Here are some studies on these topics.

Breitkreutz A., H. Choi, J.R. Sharom, L. Boucher, V. Neduva, B. Larsen, Z-Y. Lin, B-J. Breitkreutz, C. Stark, G. Liu, J. Ahn, D. Dewar-Darch, T. Reguly, X. Tang, R. Almeida, Z.S. Qin, T. Pawson, A-C. Gingras, A.I. Nesvizhskii, M. Tyers- 2010-A global protein kinase and phosphatase interaction network in yeast- Science 328: (#5981, 5/21) 1043-1046

Collins M.O- 2009-Evolving cell signals- Science 325: (#5948, 9/25) 1635-1636

Holt L.J., B.B. Tuck, J. Villén, A.D. Johnson, S.P. Gygi, D.O. Morgan- 2009-Global analysis of Cdk1 substrate phosphorylation sites provides insights into evolution- Science 325: (#5948, 9/25) 1682-1686

Jørgensen C., A. Sherman, G.I. Chen, A. Pasculescu, A. Poliakov, M. Hsiung, B. Larsen, D.G. Wilkinson, R. Kinding, T. Pawson- 2009-Cell-specific information processing in segregating populations of Eph receptor ephrin-expressing cells- Science 326: (#5959, 12/11) 1502-1509

Levy E.D., C.R. Landry, S.W. Michnick- 2010-Signaling through cooperation- Science 328: (#5981, 5/21) 983-984

Tan C.S.H., A. Pasculescu, W.A. Lim, T. Pawson, G.D. Bader, R. Linding- 2009-Positive selection of tyrosine loss in metazoan evolution- Science 325: (#5948, 9/25) 1686-1688

This article describes some of the historical aspectes of the discovery of **phosphorylation cascades**.

Catterall W.A., J.D. Scott- 2010-Edwin G. Krebs (1918-2009)- Science 327: (#5965, 1/29) 537

In addition to phosphorylation, other means of regulating protein activities by other covalent changes have been found to be used as part of post-translaltional transduction processes. Here is an example of **acetylation** of proteins used in **transduction**.

Choudhary C., C. Kumar, F. Gnad, M.L. Nielsen, M. Behman, T.C. Walther, J.V. Olsen, M. Mann- 2009-Lysine acetylation targets protein complexes and co-regulates major cellular functions- Science 325: (#5942, 8/14) 834-840

Norvell A., S.B. McMahon- 2010-Rise of the rival- Science 327: (#5968, 2/19) 964-965

Zhao S., W. Xu, W. Jiang, W. Yu, Y. Lin, T. Zhang, J. Yao, L. Zhou, Y. Zeng, H. Li, Y. Li, J. shi, W. An, S.M. Hancock, F. Fe, L. Qin, J. Chin, P. Yang, X. Chen, Q. Lei, Y. Xiong, K-L. Guan- 2010-Regulation of cellular metabolism by protein lysine acetylation- Science 327: (#5968, 2/19) 1000-1004

This article finds a role for an **ATPase** in transduction and **receptor activation**.

Cruciat C-M., B. Ohkawara, S.P. Acebron, E. Karaulanov, C. Reinhard, D. Ingelfinger, M. Boutros, C. Niehrs- 2010-Requirement of prorenin receptors and vacuolar H^+-ATPase-mediated acidification for Wnt signaling- Science 327: (#5984, 1/22) 459-463

I have found no evidence in the literature for the Ca^{+2}-pump indicated by figure 11.11 of Campbell et al. (2008) to exist. However there are **Ca^{+2}** transport systems in the **inner mitochondrial membrane**, and the matrix space is suggested to play a role in buffering Ca^{+2} concentrations and so influences its use as a **secondary messenger** by altering the amplitude and timing of pulses of this ion. Here are some studies I have found of these transport systems:

Demaurex N., D. Poburko- 2009-A revolving door for calcium- Science 326: (#5949, 10/2) 57-58

Gunter T.E., D.I. Yule, K.K. Gunter, R.A. Eliseev, J.D. Salter- 2004-Calcium and mitochondria- FEBS Letters 567: 96-102

Jiang D., L. Zhao, D.E. Clapham- 2009-Genome-wide RNAi secreen identifies Letm1 as a mitochondrial Ca^{2+}/H^+ antiporter- Science 326: (#5949, 10/2) 144-147

Signal transduction can also involve getting the proper proteins together into clusters; in protein scaffolds. Arrestin proteins have been found to play a role in this process by forming **adaptor complexes**. Another protein used in transduction is ubiquitin which is covalently added to other proteins such as histones.

Grady E.F- 2007-β-arrestin, a two-fisted terminator- Science 315: (2/2) 605-606

Scott J.D., T. Pawson- 2009-Cell signaling in space and time: Where proteins come together and where they're apart- Science 326: (#5957, 11/27) 1220-1224

Smith E., A. Shilatifarad- 2009-Histone cross-talk in stem cells- Science 323: (#5911, 1/9) 221-222

For more on the **bacterial two-component system** see:

Gunawardena J- 2010-Biological systems theory- Science 328: (#5978, 4/30) 581-582

Here are some articles related to various types of **receptors**. Some are light receptors, one describes an oxidation-state receptor, another a tyrosine phosphatase based receptor, and some deal with the functioning of G-protein coupled receptors (GPCR):

Aricescu A.R., C. Siebold, K. Choudhuri, V.T. Chang, W. Lu, S.J. Davis, P.A. van der Merwe, E.W. Jones- 2007-Structure of a tyrosine phosphatase adhesive interaction reveals a spacer-clamp mechanism- Science 317: (#5842, 8/31) 1217-1220

Cherezov V., D.M. Rosenbaum, M.A. Hanson, S.G.F. Rasmusen, F.S. Thian, T.S. Kobilka, H-J. Choi, P. Kuhn, W.I. Weis, B.K. Kobilka, R.C. Stevens- 2007-High-resolution crystal structure of an engineered human ß$_2$-adrenergic G protein-coupled receptor- Science 318: (#5854, 11/23) 1258-1265

Burgoyne J.R., M. Modhani, F. Cuello, R.L. Charles, J.P. Brennan, E. Schröder, D.D. Browning, P. Eaton- 2007-Cysteine redox sensor in PKG1α enables oxidant-induced activation- Science 317: (#5843, 9/7) 1393-1397

Gong H., B. Shen, P. Flevaris, C. Chow, S.C-T. Lam, T.A. Voyho-Yasenetskaya, T. Kozasa, X. Du- 2010-G protein subunit G$_{\alpha 13}$ binds to integrin $\alpha_{IIb}\beta_3$ and mediates integrin "outside-in" signaling- Science 327: (#5963, 1/15) 340-343

Jaokola V-P., M.T. Griffith, M.A. Hanson, V. Cherezov, E.Y.T. Chien, J.R. Lane, A.P. Ijzerman, R.C. Stevens- 2008-The 2.6 angstrom crystal structure of a human A$_{2A}$ adenosine receptor bound to an antagonist- Science 322: (#5905, 11/21) 1211-1217

Lin R., L. Ding, C. Casola, D.R. Ripoll, C. Feschotte, H. Wang- 2007-Transposase-derived transcription factors regulate light signaling in *Arabidopsis*- Science 318: (#5854, 11/23) 1302-1305

Ranganathan R- 2007-Signaling across the cell membrane- Science 318: (#5854, 11/23) 1253-1254

Rewitz K.F., N. Yamanaka, L.I. Gilbert, M.B. O'Connor- 2009-The insect neuropeptide PTTH activates receptor tyrosine kinase torso to initiate metamorphosis- Science 326: (#5958, 12/4) 1403-1405

Rosenbaum D.M., V. Cherezov, M.A. Hanson, S.G.F. Rasmussen, F.S. Thian, T.S. Kobilka, H-J. Choi, X-J. Yao, W.I. Weis, R.C. Stevens, B.K. Kobilka- 2007-GPCR engineering yields high-resolution structural insights into ß$_2$-adrenergic receptor function- Science 318: (#5854, 11/23) 1266-1273

Rubio V., X.W. Deng- 2007-Standing on the shoulders of G1GANTEA- Science 318: (#5848, 10/12) 206-207

Shen Y., A.P. Tenney, S.A. Busch, K.P. Horn, F.X. Cuascut, K. Liu, Z. He, J. Silver, J.G. Flanagan- 2009-PTP6 is a receptor for chondroitin sulfate proteoglycan, an inhibitor of neural regeneration- Science 326: (#5952, 10/23) 592-596

Slessareva J.E., H.G. Dohlman- 2006-G protein signaling in yeast: New components, new connections, new compartments- Science 314: (12/1) 1412-1413

Sussman M.R., G.N. Phillips jr.- 2009-How plant cells go to sleep for a long, long time- Science 326: (#5958, 12/4) 1356-1357

BIO 107   2010
Day 5, Lecture 12, Title: Eukaryotic Diversity I: Protists
Text Readings: Campbell et al. (2008), Chapter 28, and pgs. 538-539, 542-544, 551-553.

Topics to cover:

> **Protists as model organisms for study**
> **Attempts at classification**
> > **Some traits and examples**
> > **Cladograms and cladistics**
> **Some complications**
> > **Primary Endosymbiosis**
> > **Secondary Endosymbiosis**
> > **Horizontal versus Vertical Gene Flow**

Protists as model organisms for study
> Use as models of ourselves, of our diseases, and evolution...
> Use as model for plants
> > *Chlamydomonas*, a green alga
> > > Fig. 28.22, Chlamydomonas life cycle
> > green algae are close relatives of plants. Do photosynthesis in similar ways.
> > Grow much faster, so easier to study than oak trees...
> cilia and cystic fibrosis
> > Paramecial cilia and human epithelial cells of lung have cilia as well
> > errors in human cilia function gives cystic fibrosis, can study this in ciliates
> > > Fig. 28.11, ciliates, *Paramecium* cell structures
> jellyfish and paramecia?
> > Fig. 12.17, (Nester etal, 1995) *Paramecium* pellicle
> > > file: paramecium_pellicle.jpg
> > note trichocysts, so diverse ways to give protection at cellular level
> action potentials
> > also found in paramecia.... why? Not just in animal neurons...
> > many of the cellular traits we see in our cells also found in protists
> pathogen and parasitic studies
> > *Plasmodium* and malaria
> > > Fig. 28.10, *Plasmodium* life cycle
> > > millions of people get this illness each year.
> > > study of pathogens of us involves understanding protists

multicellularity
Came about several times. What is needed for it...?
Fig. 28.27, cellular slime mold, *Dictyostelium*, (Campbell and Reece, 2005)
One thing needed is cell to cell communication
slime mold cells secrete cAMP as a signal for aggregation
Fig. 7, (Griffin, 1994) *Dictyostelium*.jpg
How far can one species change over its life cycle?
Pfiesteria sp. (Burkholder, 1999)
A dinoflagellate, a common type of algae, involved in red tides and fish kills
Pfiesteria SEM.jpg (Burkholder 1999)
Pfiesteria life cycle options.jpg (Burkholder 1999)
Has 24 stages in life cycle, ameoboid-like to ones with cell walls!
Eat various items, bacteria to mammalian cells.
Has toxins that are volatile, kill neurons. Promotes memory loss in us.
Pfiesteria protective gear.jpg (Burkholder 1999)
Those who initially studied it suffered mental disorders.
Needs a type III biohazard facility to study!

Attemps at classification
The classification of eukaryotes has changed greatly in the past 50 years.
Will give some illustrations of types of traits seen in protists
and how to approach classification.

Some traits and examples

Defining protists is difficult, what characteristics to use? What diversity exists?
Differences in how they move
pseudopods, cilia, flagella, or sessile
with cell walls, or not   contractile vacuole
different nuclear types   photosynthetic or not
one nucleus per cell to many, coenocytic   microscopic to macroscopic
single celled to multicellular   mobile, if so how?
one cell type to many different cell types
Examples: to illustrate some of these traits
Fig. 28.3
Diatoms: mineral cell walls, photosynthetic, flagella
The cell walls fit together like glass petri dishes.
Small holes in the cell walls allow items to get to cell.
Has flagellum.
Fig. 28.16   Kelp forest: a multicellular lineage, photosynthetic
Can be hundreds of feet long. Many specialized cell types.
Some cells have flagella

Fig. 28.19   Red algae: another multicellular photosynthetic lineage
    Also many specialized cells.
    Multicellularity did evolve several times.
    lacks any cilia or flagella
    By looking at different instances of multicellularity might we
        determine what is essential to be a multicellular organism?
fig. 28.23, (Campbell and Reece, 2002)   Caulpera
    a coenocytic alga, up to meters long but all one cell!
    Has action potentials, rapid cytoplasmic streaming.
Fig. 28.11, ciliates, *Paramecium* cell structures
    No cell wall, cilia for motion. Macro and micro nuclei.
Fig. 28.11, paramecial life cycle
    macronucleus has copies of genes that are expressed and used.
    micronuclei have "stored" copies of genes, not expressed.
    contractile vacuole for osmoregulation

Current definitions are just proposals, likely to change.
    Fig. 28.3, Eukaryotic tree

Cladograms and cladistics
Cladistics is one method commonly used to try generate a hypothesis concerning relations.
Attempts to use changes in characters to determine relationships.
    Resulting cladogram is a hypothesis of relationships.
    Fig. 26.11, making a cladogram
Needed to make a cladogram:
    Must know many character states of species, often arranged in a table.
    Define an "outgroup" to anchor the cladogram.
        It should have "ancestral" state for each of the character states being used.
    If the characters show up in the lineage, the ancestral state should lack them.
    Propose order that character states shifted, and arrange lineage branching
Ask students to consider making a cladogram of selected groups of protists.
    And consider how to arrange them in a cladogram.
    Chlamydomonas, paramecium, red algae, kelp

	Mobile cells?	multicellular?	Photosynthetic?	eukaryotic?
Chlamydomonas	y	n	y	y
paramecium	y	n	n	y
red algae	n	y	y	y
kelp	y	y	y	y

    Obviously this makes a hypothesis, need to test it with other data...
    (Note, above does not have a well defined outgroup... propose one?)

Some complications

As argue over character states and order of changes

get new arrangements of classifications....

Fig. 28.3, eukaryotic phylogeny

This figure suggests nice distinct lineages

some argue for > 27 kingdoms just of "protists"

We do not know which traits are relevant, and classification is still tentative

Other than ignorance of traits, other factors play a role in complicating thing...

Traits can be lost as well as gained, traits can evolve several times

consider multicellularity:

evolved once, so all related?

or evolved several times, then how many times?

was it lost from any lineage?

Primary Endosymbiosis

Primary endosymbiosis, produces some organelles in eukaryotic cell

Fig. 28.6, (Campbell and Reece, 2002) domain phylogeny (with some endosymbiosis)

Fig. 26.22   This suggests only a few events of horizontal gene flow.

Fig. 28.7, (Campbell and Reece, 2002) domain phylogeny (more endosymbiosis...)

note problem for relationship reconstruction this creates...

Traits are based on genes, this passes prokaryotic genes into a eukaryotic cell

Won't this eukaryotic cell then look, at the molecular level more like a prokaryote?

Fig. 26.13, primary endosymbiosis (Campbell and Reece, 2005)

Is this an ancient event only, or does it still happen today?

Work by Jeon (1970s)

Amoeba, has infectious bacterium

nuclear transfer, cytosolic transfer procedure

where is the essential genetic material?

"chimera" and idea of sudden evolutionary shift....?

Jeon etal. (1976)

Nucleus from an infected cell moved into the cytoplasm of a de-nucleated

uninfected cell will only produce a viable cell if some cytosol from the

infected cell is also transfered. Turns out infecting agent, X-bacterium,

in that cytosol was critical.

Strain of amoebae infected in 1967, about 40000 bacteria per cell. Some

individuals survived.

Bacteria live in vesicles derived from food vacuoles, but not acidic, symbiosomes.

Found bacteria could no longer live independently, and infected cell needed

something provided by the bacterium.

So genes from host amoeba now in bacterium,

genes from bacterium now in host amoeba's nucleus.

so an endosymbiosis established!

Note work by Small etal. (1994), how parasites can live intracellularly

Secondary Endosymbiosis

Fig. 28.5, (Campbell and Reece, 2002) secondary endosymbiosis

Here a eukaryotic cell is taken up by another eukaryotic cell.
     This can move genes across lineages of eukaryotes?
Fig. 28.3, (Campbell and Reece, 2005)   primary, secondary, tertiary... endosymbiosis
     So get endosymbiosis and genes to flow between eukaryotes as well...

Things not shown, what happened to other organelles? McFadden etal. (1994)
     amoeba ate an alga: nucleoid, with three chromosomes very small
          surrounded by two membranes with pores.

Horizontal versus Vertical Gene Flow

Vertical gene flow: parents to offspring, across generations but within one lineage.
     the assumed pattern.
     This is what we normally assume when we try to reconstruct relationships.
Horizontal gene flow: From one lineage to another. Ex. bacteria to protists.
     makes things look more closely related than justified by traits.
     endosymbiosis often associated with horizontal gene flow.
          ex: we have bacterial-like genes in our nuclei from the
               bacterium that became our mitochondria...

Does this happen today?
     Work by Kimura and Nishihara
     note movement of genes between nucleus and organelles
     if enough move it becomes an obligate relationship

Revisit the model
     Fig. 28.7, (Campbell and Reece, 2002) domain phylogeny (more endosymbiosis...)
     Note that have endosymbiosis amongst eukaryote lineages.
          This makes defining kingdoms even more difficult
          You want to use traits that define the lineage,
               but what if it jumped into it from another lineage?
     Recall we also have endosymbiosis of plastids to plant lineage,
          and mitochondria into animal lineage
     Many algal lineages have acquired endosymbiotic organelles separately.
     Fig. 28.3, protist phylogeny
          This is still a hypothesis, and is very likely to be revised
          Some parts are fairly set...

If Time: On MBOTC CD: 25.2, *Listeria* parasites with cytoskeleton
     24.5, killer T cell; 1.2, Amoeba moving; 1.3, Eutreptiella

Objectives:

What are examples of characteristics shared by humans and unicellular protists? Consider how a study of these protists can be used to explore the nature of cells in humans.

What is the distinction between primary endosymbiosis and secondary endosymbiosis? What are examples of organisms which are thought to have come about by each type of endosymbiosis?

How is paramecial sex different from our own? What role does the macronucleus and the micronuclei play in their lives?

Be able to describe how you could determine whether endosymbiosis has occurred and whether it is primary or secondary endosymbiosis.

How has the existence of various types of endosymbiosis complicated attempts to classify organisms according to their evolutionary history? Be able to distinguish vertical versus horizontal gene flow and describe how knowing the past occurrences of such events is important for attempts to reconstruct past evolution.

Given a hypothetical cladogram be able to discern the relationships shown in it and the implied order of appearance of traits it shows.

Know general traits of the following groups [*Euglena* sp., *Paramecium* sp., *Pfiesteria* sp., *Dictyostelium* sp. (Slime mold), Diatoms, *Chlamydomonas* sp., Kelp]. Some important traits to consider include: The presence and nature of any cell walls. Coenocytic species. The presence of macro- and micro-nuclei. Whether that group is autotrophic or heterotrophic. Specific means of locomotion. Be able to arrange these groups using their traits in a hypothetical phylogenetic tree showing which traits appear at the various branch points.

For review see self-quiz questions #2 and 4 of chapter 28, and #4 of chapter 26.

Needed overheads and items:

Fig. 28.22, *Chlamydomonas*
Fig. 28.11, ciliates, *Paramecium* cell structures
Fig. 12.17, (Nester etal, 1995) *Paramecium* pellicle, file: paramecium_pellicle.jpg
Fig. 28.10, *Plasmodium* life cycle
Fig. 28.27, (Campbell and Reece, 2005) cellular slime mold, *Dictyostelium*
Fig. 7, (Griffin, 1994) *Dictyostelium*.jpg
Pfiesteria SEM.jpg (Burkholder 1999)
Pfiesteria life cycle options.jpg (Burkholder 1999)
Pfiesteria protective gear.jpg (Burkholder 1999)
Fig. 28.3   Diatoms
Fig. 28.16   Kelp forest
Fig. 28.19   Red algae
fig. 28.23, (Campbell and Reece, 2002)   Caulpera
Fig. 28.11, ciliates, *Paramecium* cell structures
Fig. 28.11, paramecial life cycle
Fig. 28.3, Eukaryotic diversity
Fig. 26.11, making a cladogram
Fig. 28.3, eukaryotic phylogeny (no endosymbiosis)
Fig. 28.6, (Campbell and Reece, 2002) domain phylogeny (with some endosymbiosis)
Fig. 26.22, horizontal gene flow
Fig. 28.7, (Campbell and Reece, 2002) domain phylogeny (more endosymbiosis...)
Fig. 26.13, (Campbell and Reece, 2005) primary endosymbiosis
Fig. 28.5, (Campbell and Reece, 2002) primary and secondary endosymbiosis
Fig. 28.3, (Campbell and Reece, 2005) origin of plastid diversity
Fig. 28.7, (Campbell and Reece, 2002) domain phylogeny (more endosymbiosis...)
Fig. 28.3, eukaryotic phylogeny

If time:
On MBOTC CD: 25.2, *Listeria* parasites with cytoskeleton
On MBOTC CD: 24.5, quicktime movie, killer T-cell
On MBOTC CD: 1.2, quicktime movie, Amoeba moving
On MBOTC CD: 1.3, quicktime movie, Eutreptiella

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002-<u>Molecular biology of the cell. Cell Biology Interactive CD</u>. Movies 25.2. Garland Science Press. N.Y., N.Y.

Burkholder J.M- 1999-The lurking perils of *Pfiesteria*- Scientific American 281: (#2) 42-49

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008bb-Protists. Chapter 28. Pages 575-599, and pgs. 538-539, 551-553, and 542-544. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition, Fig. 26.13, 28.3, 28.27. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figures 28.5, 28.6, 28.7, 28.23. Benjamin Cummings Press. San Francisco, CA.

Griffin D.H- 1994-<u>Fungal Physiology</u>, 2nd edition. Figure 7. Wiley-Liss Press. N.Y.

Jeon K.W- 1991-Amoeba and x-bacteria: Symbiont acquisition and possible species change- pgs 118-131 of <u>Symbiosis as a source of evolutionary innovation: Speciation and morphogenesis</u>. L. Margulis and R. Fester editors. MIT Press, Cambridge, MA.

Jeon K.W- 1972-Development of cellular dependence on infective organisms: Microsurgical studies in Ameobas- Science 176: (June 9) 1122-1123

Jeon K.W., M.S. Jeon- 1976-Endosymbiosis in *Amoebae*: Recently established endosymbionts have become required cytoplasmic components- Journal of Cellular Physiology 89: 337-344

Kimura K., T. Kosaka, T. Takahashi- 1992-Reestablishment of symbiotic association between algae-free *Paramecium bursaria* and symbiotic algae from green paramecia- Zoological Science (Tokyo) 9: (#6) 1280

McFadden G.I., P.R. Gilson, C.J.B. Hofmann, G.J. Adiock, U-G. Maier- 1994-Evidence that an amoeba acquired a chloroplast by retaining part of an engulfed eukaryotic algae- PNAS 91: 3690-3694

Nester E.W., C.E. Roberts, M.T. Nester- 1995-<u>Microbiology: A human perspective</u>. Fig. 12.17. W.C. Brown Publishing. Dubuque, Iowa.

Nishihara N., T. Takahashi, T. Kosaka, H. Hosoya- 1996-Characterization of symbiotic algae-free strains of *Paramecium bursaria* produced by the herbicide paraquat- Journal of Protozoology Research 6: 60-67

Nishihara N., S. Horiike, T. Takahashi, T. Kosaka, Y. Shigenaka, H. Hosoya- 1998-Cloning and characterization of endosymbiotic algae isolated from *Paramecium bursaria*- Protoplasma 203: 91-99

Small P.L.C., L. Ranakrishnan, S. Falkow- 1994-Remodeling schemes of intracellular pathogens- Science 263: 637-639

Related issues:

Here are some articles that deal with the classification of various **protistian** groups:

Andersen R.A- 1998-What to do with protista?- Australian Systematic Botany- 1: (#2) 185-201

Andersen R.A- 2004-Biology and systematics of heterokont and haptophyte algae- American Journal of Botany 91: (10) 1508-1522

Hackett J.D., D.M. Anderson, D.L. Erdner, D. Bhattacharya- 2004-Dinoflagellates: A remarkable evolutionary experiment- American Journal of Botany 91: (10) 1523-1534

Lewis L.A., R.M. McCourt- 2004-Green algae and the origin of land plants- American Journal of Botany 91: (10) 1535-1556

Perasso R., A. Baroin, L.H. Qu, J.P. Bachellerie, A. Adoutte- 1989-Origin of the algae- Nature 339: (May 11) 142-144

Ragan M.A- 1998-On the delineation and higher-level classification of algae- European Journal of Phycology 33: 1-15

Saunders G.W., M.H. Hommersand- 2004-Assessing red alga supraordinal diversity and taxonomy in the context of contemporary systematic data- American Journal of Botany 91: (10) 1494-1507

A review of techniques used to determine **classification** of groups of life and how the classifications have changed:

Doolittle W.F- 2000-Uprooting the tree of life- Scientific American 182: (#2) 90-95

Embley T.M., R.P. Hirt, D.M. Williams- 1994-Biodiversity at the molecular level: The domains, kingdoms and phyla of life- Philosophical Transactions of the Royal Society of London (B) 345: 21-33

Grant V- 2003-Incongruence between cladistic and taxonomic systems- American Journal of Botany 90: (#9) 1263-1270

Kumar S., A. Rzhetsky- 1996-Evolutionary relationships of eukaryotic kingdoms- Journal of Molecular Evolution 42: 183-193

One reason for studying the protists is that they are useful **model systems** for studying aspects of agricultural and human systems.   Here are some examples. One deals with study of **carbohydrate metabolism**, while the other deals with hydrogen production by green alga as a means to create **biofuels**.

Hicks G.R., C.M. Hironaka, D. Dauville, R.P. Funke, C.D. Hulst, S. Waffenschmidt, S.G. Ball- 2001-When simpler is better. Unicellular green algae for discovering new genes and functions in carbohydrate metabolism- Plant Physiology 127: (#4) 1334-1338

Melis A., T. Happe- 2001-Hydrogen production. Green algae as a source of energy- Plant Physiology 127: (#3) 740-748

Some articles touching on **endosymbiosis**:

Dagan T., W. Martin- 2009-Seeing green and red in diatom genomes- Science 324: (#5935, 6/26) 1651-1652

Keeling P.J- 2007-Deep questions in the tree of life- Science 317: (#5846, 9/28) 1875-1876

Keeling P.J- 2004-Diversity and evolutionary history of plastids and their hosts- American Journal of Botany 91: (#10) 1481-1493

Margulis L., D. Sagan- 1997- Slanted truths: Essays on Gaia, symbiosis and evolution- Copernicus Press, N.Y. 368 pages.

McNeil P.L., P.J. McAuley- 1984-Lysosomes fuse with one half of alga-bearing phagosomes during the reestablishment of the european green hydra symbiosis- Experimental Zoology 230: (#3) 377-386

Moustafa A., B. Beszterl, U.G. Maier, C. Bowler, K. Valentin, D. Bhattacharya- 2009- Genomic footprints of a cryptic plastid endosymbiosis in diatoms- Science 324: (#5935, 6/26) 1724-1726

Nakabachi A., A. Yamashita, H. Toh, H. Ishikawa, H.E. Dunbar, N.A. Moran, M. Hattori- 2006-The 160-kilobase genome of the bacterial endosymbiont *Carsonella*- Science 314: (10/13) 267

Newton I.I.G., T. Wayke, T.A. Auchtung, G.F. Dilly, R.J. Dutton, M.C. Fisher, K.M. Fontanez, E. Lau, F.J. Stewart, P.M. Richardson, K.W. Barry, E. Saunders, J.C. Detter, D. Wu, J.A. Eisen, C.M. Caranaugh- 2007-The *Calyptogena magnifica* chemoautotrophic symbiont genome- Science 315: (2/16) 998-1000

Trémouillaux-Guiller J., T. Rohr, R. Rohr, V.A.R. Huss- 2002-Discovery of an endophytic alga in *Ginkgo biloba*- American Journal of Botany 89: (#5) 727-733

So how do **diatoms** live in glass-houses? This article describes some aspects of how their glass cell wall structure is produced.

Zurzol C., C. Bowler- 2001-Exploring bioinorganic pattern formation in diatoms. A story of polarized trafficking- Plant Physiology 127: (#4) 1339-1345

For more on **red-tide toxins** made by protista see this study of the diverse types of items detected. Some of these molecules turn out to leads for possible drug development.

Potera C- 2007-Florida red tide brews up drug lead for cystic fibrosis- Science 316: (6/15) 1561-1562

How a **bi-flagellate green alga** swims, and how it varies its swimming patterns in adaptive ways, are considered in these papers.

Polin M., I. Tuval, K. Drescher, J.P. Gollub, R.E. Goldstein- 2009-*Chlamydomonas* swims with two "gears" in a eukaryotic version of run-and-tumble locomotion- Science 325: (#5939, 7/24) 487-490
Stocker R., W.M. Durham- 2009-Tumbling for stealth?- Science 325: (#5939, 7/24) 400-402

Cellular slime molds have interesting behaviors and an ability to organize into multicellular structures. One group has studied their ability to self-organize as a model for how to design a metropolitian transportation system!

Marwan W- 2010-Amoeba-inspired network design- Science 327: (#5964, 1/22) 419-420
Tero A., S. Takagi, T. Saigusa, K. Ito, D.P. Bobber, M.D. Fricker, K. Yumiki, R. Kobayashi, T. Nakagaki- 2010-Rules for biologically inspired adaptive network design- Science 327: (#5964, 1/22) 439-442

This study describes how **sexual dimorphism** may have evolved in the green alga, *Volvox*.

Ferris P., B.J.S.C. Olson, P.L. De Hoff, S. Douglass, D. Casero, S. Prochnik, S. Geng, R. Rai, J. Grimwood, J. Schmutz, K. Nishii, T. Hamaji, H. Nozaki, M. Pellegrini, J.G. Umen- 2010-Evolution of an expanded sex-determining locus in *Volvox*- Science 328: (#5976, 4/16) 351-354

BIO 107   2010
Day 5, Lecture 13, Title: Being Multicellular
Text Readings: Campbell and Reece (2008), chapter 40, and pgs. 118-121, 517-518, 565.

Topics to cover:

> **Essential functions**
> **Diffusion**
> **Bacterial options**
> **Eukaryotic options**
> **New issues when multicellular**
> **Tissues that help meet needs (animals...)**
> **Homeostatic mechanisms: Temperature regulation**
> **Summary**

Essential functions

Whether multi- or single- celled some key functions are seen.
> But these may be altered in multicellular organisms?

Consider, which of the following would be altered the most?
> If there are benefits to being multicellular, then it can selected for.
> Though in some conditions it is better to be single celled...

And recall, that each cell in a multicellular organism still has to do most of these.
> So most of the cellular processes are retained, with a few exceptions...

Some functions:

Table 40.1   Organ systems, mammalian
> Consider how increase in body size results in the need for these systems.
> Exceed diffusion limits, and need a circulatory system? etc...

Will look at some of the ways the above are modified as go from single to multicelled state

Diffusion and Options of Single Cells.

Diffusion limitations: Movement of molecules down energy gradients.
> In water occurs over distances of millimeters. In air over distances of centimeters.
> Fig. 40.3, contact with environment
> Faster if: (and faster if some things can be altered to promote it)
> > Molecule is smaller rather than larger
> > > so exchange small items not larger
> > > (Relate this to covalent modifications, and digestion?)
> > Energy (i.e. Concentration) gradient is larger rather than smaller
> > > (use transport systems and bulk flow to enhance gradient?)
> > Distance over which it is done is shorter rather than further
> > > (alter shapes of cells and ECM to change thickness of layers)
> > Done over more area rather than over a small area
> > > (alter total area used and change cellular shape and SA/vol ratio)
> Note that the a cell's metabolic rate may be limited by the ability to take up items,
> > acquired by diffusion. Cells must deal with this issue...

In some cases wish to enhance exchange rates, in other cases wish to limit it.
Consider dormancy, a chicken egg, life in a desert.
these all involve avoiding the loss of items?
and so diffusion should be limited?

Bacterial options
I am arguing that the traits we see in multicellular organisms are often adaptations of ones
already found in single celled organisms.
Before multicellularity, need diversity in single celled organsims to give a "toolkit"?
Many of these options relate to diffusion limits, and later are needed for multicellularity...
Small size of cell: this allows diffusion to be sufficient to supply needs
fig. 6.6, prokaryotic cell
fig. 27.2, bacterial shapes
Given diffusion limits, staying small is a viable option. Avoid circulatory needs?
Can limit demands from environment, by going dormant
fig. 27.9, endospores
This collapses the concentration gradient, as no use of certain items.
And it allows avoidance of stressful environmental conditions, allows dispersal, etc
New compartments
fig. 27.7, internal membranes
Notice how this alters the surface area.
use new membrane regions for new functions, specializations
multicellularity will open up new types of specializations....
but first would expect to have specializations in various regions in one cell
Means to interact with environment and others
fig. 27.5, fimbriae
has receptors on surfaces for contacts, sensing the environment
bacterial communities
fig. 27.15, biofilms and metabolic cooperation
separate metabolic traits amongst individuals
shared to carryout metabolism as a group,
an analogy for a multicellular organism?
signals sent between them (Holloway, 2004) called quorum sensing
When signals exceed a certain concentration,
see shift in physiology of population members.
ex. bioluminescence only when > a critical pop. density
ex. pathogen, *Salomonella*, releases toxin only at > critical pop. density.
senses and cell-to-cell talking as essential for multicellularity...

Multicellular bacteria
    Multicellularity has evolved several times in bacteria as well
    Fig. 27.14 *Anabaena*
    cellular specialization is seen, nitrogen fixing cells
            so specialization of metabolism here,
            this implies changes in gene expression patterns
    cells must hold on to other cells in the group
            cell-to-cell adhesion either to each other or to an ECM is needed
Bacteria are mainly opportunists.
    Under good conditions grow rapidly, under poor conditions go dormant
    But evolved before eukaryotes and traits anticipate ones needed for multicellularity

    So many of the traits needed for eukaryotes to become multicellular may have been inherited
from their prokaryotic ancestors, others built from this base.
    Sensing of environment
    Metabolic cooperation
    Cellular specializations
    Cell-to-cell attachments and signaling.... etc...

Eukaryotic options
    Recall the diverse single celled protists we have considered...
    Larger size, so begin to see diffusion limits
            Fig. 6.27
            Hense cytosolic streaming via cytoskeleton
            slower in growth rates than prokaryotes
    Via endomembrane system and endosymbiosis have many new compartments
            Fig. 6.9, animal cell
            this gives more specializations in regions of cell, new cell surface shapes
            so must express different genes for proteins to be used in different places...
            option of phagocytosis, rare in prokaryotes, produces an internalized exterior...
    Allows for distinct internal compartments, differing in SA/VOL ratios, functions,
            and can be used in homeostasis of cytoplasm...

New issues when multicellular
    Cells must be physically connected to other cells, or to an external matrix
            cell-to-cell junctions
                    Fig. 6.32   intercellular junctions, animals
                    can make new compartments via tight junctions
            Extracellular matrix and cell walls
                    Fig. 6.28   plant cell wall
                    Fig. 6.30   ECM of animals
                    gives items for cells to hold onto for anchorage, or be held within

New extracellular compartments may be formed
      fig. 32.4, origin of animals (Campbell and Reece, 2005)
      note internal chamber, and when folded it can act as a digestive cavity...
      need cell-cell attachment and control of shape over many cells?
          so coordination between cells becomes essential.
      Internal environment versus external now selects for specialization?
Cells must communicate with each other; recall signal, receptors, transduction, responses
      could send cell-cell signals via cytoplasmic connections
          gap junctions, plasmodesmata
          Fig. 6.32   intercellular junctions, animals
          Fig. 6.31, plasmodesmata
      could use extracellular signals
          slime molds aggregate up a concentration gradient of cAMP
          Fig. 28.25   cellular slime mold life cycle
          video - L18-slime4-credits - Slime mold culture
      Some cells might send different signals,
          consider the endocrine glands and hormones?
Physical laws can dictate limits when become larger in size
      Diffusion limits can constrain shape of organism
          fig. 40.3, contact with the environment
          may also use circulatory systems to deal with such issues?
      physical laws can dictate morphology of an organism
          cell-cell attachments have to withstand physical shear forces
             Such as wind, wave actions, etc...
          Organs have to connect to organs strongly enough
             This gets into biomechanics.
          Similar physical conditions can result in similar solutions
             fig. 40.2
             example of convergence for swimming
Individual cells also must surrender certain options
          migrate, divide, or express all of their genes?
      Benefit from the rest of organism by
          control of water content, temperature, mineral content, etc...
      Organism, a long term item, so has benefits and costs in new ways?
          benefit is that can disperse better, reproduce in new ways?
             can survive in new habitats, use new food sources, etc...
          who gets to reproduce? tyranny of the organism over cells?
          somatic cells help their genetic "relatives" in the organism to reproduce,
             but not every cell gets to reproduce themselves?
      Also need to defend the organism from pathogens.
          So specialization of new defenses,
          and kill off somatic cells who violate these new rules... consider cancers.

Tissues that help meet needs (animals...)

        Being multicellular can specialize...

        Tissue... "... groups of cells of similar appearance and a common function."

                                  (Campbell et al., 2008; pg. 855)

        In animals a tissue also involves being held together by extracellular matrix

                this is called a "basement membrane" (not a cellular membrane).

        Cells in different animal tissues aggregate due to specific surface binding proteins

                such as CAMs (Cell Adhesion Molecules)

Review basic tissues in animals.

nervous

        Fig. 48.2 (Campbell and Reece, 2002)   Neuron

        Fig. 49.2   various animal nervous systems

        Neuron and other cells (glial cells, etc) in nervous system.

        Do rapid signal transduction in the organism. Also often secrete chemical signals.

        Large size requires intraorganismal communication.

muscle

        Fig. 40.5   Muscle tissue

        Skeletal, smooth, cardiac, and also a fourth type in mammary glands...

        Binds to ECM, binds to other cells. Force generation.

        Uses cellular cytoskeletal elements to generate force.

        A large sized organism alters its shape, and generates force for movement.

epithelial

        Fig. 40.5   Epithelial tissue

        Create barriers, layers.

        Some are barriers to block exchanges. So stratified with many layers.

                What does this do to the rate of diffusion?

        Some are layers over which want exchange. Lungs? Intestinal lining? Capillaries?

                So simple, with just one layer.

connective

        Fig. 40.5   Connective tissue

        The extracellular matrix is what many cells latch on to to shape tissues, organs, etc.

        This tissue makes the ECM, cells secrete the proteins/minerals that makes it up.

        Loose connective tissue around tissues/organs, so can move organs in our gut...

        Fibrous connective, gives strong one directional strength. Tendons, ligaments.

        Cartilage. Dense surface coating of bones, or giving shape to sense organs (nose, ear).

        Bone. A mineral matrix. Made and modified by cells.

        Fat is also a connective tissue. As is blood.

Organs, such as the stomach, have several tissues together

        Fig. 40.6, (Campbell and Reece, 2005)   Stomach lining,

        Then have organ systems, such as the digestive tract...

Homeostatic mechanisms: Temperature regulation

Both cells and multicelled organisms must do homeostasis of many items.

But multicelled organisms, being big, have the option of temperature regulation.

Single cells can do this as populations.

(i.e. bacterial population can alter temperature)

What is a benefit of temperature control? What is the cost? This cost implies what?

Endotherms regulate their internal body temperatures in a set range. Its expensive.

Need feedback systems, focus here on control of temperature for homeostasis,

but analogous to other items...

Involves signals, reception, transduction and response mechanisms

but now done between cells in an organism rather than within one cell

Fig. 45.5   chemical signaling

cells specialize for sending signals, others to move the chemical, others to receive

so needs receptors and response systems

positive versus negative feedback control systems.

Positive feedback systems run to one extreme, not used in homeostasis..

Can get reasonable control with two counteracting negative feedback systems

Fig. 40.8   Negative feedback

example of regulation of room temperature

consider set points, and their use for negative feedback

Temperature sensing by cells in hypothalamus...

Fig. 40.16 Hypothalamus thermal setpoint example

So proteins in the hypothalamus acts as a temperature sensor, how?

sensor, signal transduction, response systems all internal

some internal signals are via nervous system, others via hormones

Transduction of information internally via hormones and nervous impulses

glandular

Fig. 45.10   Human endocrine glands

nervous

Fig. 49.4   vertebrate nervous system

These, and others, are the sort of systems needed to be multicellular

So would expect such chemical and electrical signals were used by a single cells first...

Responses/options. The signals go to other cells in the body, the do the response.

In this case to change temperature of the body there might be:

sweat gland activation

fig. 40.11

altering blood flow to surface capillaries, via vasoconstriction or diliation

fig. 40.12 counter current heat exchangers

fig. 40.16 thermoregulation in large fish (Campbell and Reece, 2005)

note counter-current system in bird leg... see how it retains heat?

Change in metabolic rate
  shivering, or use of brown fat for thermogenesis
  being and ectotherm vs. endotherm, changing the demands
  fig. 40.7, endotherm vs. ectotherm
  Endotherms are burning up food, via respiration, to make heat.
   That organic matter could be used for growth instead?
   Adds extra cost to living in a certain environment?
  If costs too much then hibernate?
  fig. 40.21, hibernation of squirrels
behavioral changes
  for cooling or warming
  fig. 40.18, elephant evaporative cooling (Campbell and Reece, 2005)
  fig. 40.13, thermoregulatory behavior in dragonfly
  fig. 40.15, preflight warmup in hawkmoth
  fig. 40.10, behavioral heat exchange

Summary

So just for temperature regulation
  see many options
  several control mechanisms
  and several response options.
So different species can vary in how this is done....
But diffusion and physical laws put limits on what can be achieved
  specialized structures must help the organism deal with these limits....
  that allows the organism to live in habitats other cannot, a benefit...
So in an animal see various organ systems that have roles in meeting organismal needs.
  Fig. 40.4, basic animal
  Every function of these organ systems, and their cells and tissues, has an evolutionary
   history. We expect past single celled organisms to show similar traits.
   Thus study of single celled organisms informs our understanding of the
    multicelled organisms. This is unity in biology, and job security!
We will look at various organ systems over many of the following lectures.
  But remember that single cells still have to survive in the organism, and still
   have to carry out their functions and deal with local diffusion limits.

Objectives:

Be able to describe several major challenges that organisms must meet if they are to be viable multicellular organisms that they did not face as single celled organisms. What are some solutions that life has evolved that meet these challenges? Look for characteristics of single-celled organisms that could be used to meet some of the challenges of a multi-celled organism. What are some traits found in prokaryotes, and what are others found in single-celled eukaryotes, that can be used by multicelled organisms to address their needs? What are some things that multicelled organisms have to worry about that most single celled organisms do not, and what are some benefits multicelled organisms gain in being multicellular?

Describe factors that can alter the rate of diffusion of an item. What are several ways that an organism can modify itself or its surroundings to enhance the total amount of items that diffuse up to its cells? Conversely how might it make a better barrier so that it can limit loses due by diffusion?

Be able to describe homeostasis in animals. What would be an example of homeostasis in a non-animal species? What are some cellular and molecular mechanisms that must be present for homeostasis to be accomplished? What is a negative feedback system, and what is a counter current exchange system? Be able to describe examples of the uses of these in an animal's temperature regulation. Be able to describe various examples of types of sensing, signal transduction, and responses that are often used as an animal carries out its temperature homeostasis.

Be able to describe the major tissues found in animals and their general functional roles. Relate them to how they help the organism meet challenges unique to being multicellular. What must be present to have an animal tissue?

Self-quiz questions #1, 3, and 6 of chapter 40 might be helpful for review.

Needed overheads and items:

Table 40.1 Organ systems, mammalian

Fig. 40.3, contact with environment

fig. 6.6, prokaryotic cell

fig. 27.2, bacterial shapes

fig. 27.9, endospores

fig. 27.7, internal membranes

fig. 27.5, fimbriae

fig. 27.15, biofilms and metabolic cooperation

Fig. 27.14 *Anabaena*

Fig. 6.27, cytoplasmic streaming and cytoskeleton

Fig. 6.9, animal cell

Fig. 6.32 intercellular junctions, animals

Fig. 6.28 plant cell wall

Fig. 6.30 ECM of animals

fig. 32.4, origin of animals (Campbell and Reece, 2005)

Fig. 6.32 intercellular junctions, animals

Fig. 6.31, plasmodesmata

Fig. 28.25 cellular slime mold life cycle

video - L18-slime4-credits - Slime mold culture

fig. 40.3, contact with the environment

fig. 40.2, convergenes of swimmers

Fig. 48.2 Neuron (Campbell and Reece, 2002)

Fig. 48.2 various animal nervous systems

Fig. 40.5 Muscle tissue

Fig. 40.5 Epithelial tissue

Fig. 40.5 Connective tissue

Fig. 40.6 Stomach lining (Campbell and Reece, 2005)

Fig. 45.5 chemical signaling

Fig. 40.8 Negative feedback

Fig. 40.16 Hypothalamus thermal setpoint example

Fig. 45.10 Human endocrine glands

Fig. 49.4 vertebrate nervous system

fig. 40.11 mammalian skin

fig. 40.12 counter current heat exchangers

fig. 40.16 thermoregulation in large fish (Campbell and Reece, 2005)

fig. 40.7, endotherm vs. ectotherm

fig. 40.21, hibernation of squirrels

fig. 40.18, elephant evaporative cooling (Campbell and Reece, 2005)

fig. 40.13, thermoregulatory behavior in dragonfly

fig. 40.15, preflight warmup in hawkmoth

fig. 40.10, behavioral heat exchange

Fig. 40.4, basic animal

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Basic priniples of animal form and function. Chapter 40. Pages 852-874, and 517-518, 565. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition, Figures 32.4, 40.6, 40.16, 40.18. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 6th edition, Fig. 48.2.   Benjamin Cummings Press. San Francisco, CA.

Holloway M- 2004-Talking bacteria- Scientific American 290: (#2) 34-35

Related issues:

For another view of **temperature regulation** in mammals and birds see:

Ishibashi J., P. Seale- 2010-Beige can be slimming- Science 328: (#5982, 5/28) 1113-1114

Sapor C.B- 2006-Life, the universe, and body temperature- Science 314: (11/3) 773-774

Tattersall G.J., D.V. Andrade, A.S. Abe- 2009-Heat exchange from the toucan bill reveals a controllable vascular thermal radiator- Science 325: (#5939, 7/24) 468-470

Vegiopoulos A., K. Müller-Decker, D. Strzoda, I. Schmitt, E. Chichelnitskiy, A. Ostertag, M.B. Diaz, J. Rozman, M.H. de Angelis, R.M. Nüsing, C.W. Meyer, W. Wahli, M. Klingenspor, S. Herzig- 2010-Cyclooxygenase-2 controls energy homeostasis in mice by *de novo* recruitment of brown adipocytes- Science 328: (#5982, 5/28) 1158-1161

Of course, the imposed **cooling** of the tissues of endotherms is often studied for use in treatments. Here is a short article on cooling of organs that normally do not get cooled:

Couzin J- 2007-The big chill- Science 317: (#5839, 8/10) 743-745

We discussed aspects of homeostasis of temperature regulation. Here are additional articles relating to **homeostasis** in other systems in the multicellular body. In many cases, as in obesity or blood pressure regulation, we notice the lack of proper homeostasis:

Flier J.S., E. Maratos-Flier- 2007-What fuels fat- Scientific American 297: (#3, Sept.) 72-81

Hartzell H.C- 2007-The stress of relaxation- Science 317: (#5843, 9/7) 1331-1332

Hawkins P.T., L.R. Stephens- 2007-PI3Kγ is a key regulator of inflammatory responses and cardiovascular homeostasis- Science 318: (#5847, 10/5) 64-66

Imaizumi T., S.A. Kay, J.I. Schroeder- 2007-Daily watch on metabolism- Science 318: (#5857, 12/14) 1730-1731

Mueller S.N., K.A. Hosiawa-Meagher, B.T. Konieczny, B.M. Sullivan, M.F. Bachmann, R.M. Locksley, R. Ahmed, M. Matloubian- 2007-Regulation of homeostatic chemokine expression and cell trafficking during immune responses- Science 317: (#5838, 8/3) 670-674

Semenza G.L- 2007-Life with oxygen- Science 318: (#5847, 10/5) 62-64

Taguchi A., L.M. Wartschow, M.F. White- 2007-Brain IRS2 signaling coordinates life span and nutrient homeostasis- Science 317: (#5836, 7/20) 369-372

White D- 2000-Homeostasis- Chapter 15, pgs. 384-397, in, The Physiology and Biochemistry of Prokaryotes. 2nd edition. Oxford University Press, N.Y.

Wisse B.E., F. Kim, M.W. Schwartz- 2007-An integrative view of obesity- Science 318: (#5852, 11/9) 928-929

Recently many **artificial tissues** have been made. Here are articles that deal with the production of replacement parts.

Klein J- 2009-Repair or replacement - A joint perspective- Science 323: (#5910, 1/2) 47-48

Service R.F- 2008-Coming soon to a knee near you: Cartilage like your very own- Science 322: (#5907, 12/5) 1460-1461

To be **multicellular** the cells in an organism must connect and overcome many issues. Here are some articles touching on these topics:

Bakal C., J. Aach, G. Church, N. Perrimon- 2007-Quantitative morphological signatures define local signaling networks regulating cell morphology- Science 316: (#5832, 6/22) 1753-1756

Bonner J.T- 1998-The origins of multicellularity- Integrative Biology 1: (#1) 27-36

Engler A.J., P.O. Humbert, B. Wehrle-Haller, V.M. Weaver- 2009-Multiscale modeling of form and function- Science 324: (#5924, 4/10) 208-212

Evans E.A., D.A. Calderwood- 2007-Forces and bond dynamics in cell adhesion- Science 316: (5/25) 1148-1153

Galbraith C.G., K.M. Yamada, J.A. Galbraith- 2007-Polymerizing actin fibers position integrins primed to probe for adhesion sites- Science 315: (2/16) 992-995

Holt C.E., S.L. Bullock- 2009-Subcellular mRNA localization in animal cells and why it matters- Science 326: (#5957, 11/27) 1212-1216

Hu K., J. Lin, K.T. Applegate, G. Danuser, C.M. Waterman-Storer- 2007-Differential transmission of actin motion with in focal adhesions- Science 315: (1/5) 111-115

Montell D.J- 2008-Morphogenetic cell movements: Diversity from modular mechanical properties- Science 322: (#5907, 12/5) 1502-1505

Scheiermann C., P. Meda, M. Aurrand-Lions, R. Madani, Y. Yiangou, P. Coffey, T.S. Salt, D. Ducrest-Gay, D. Caille, O. Howell, R. Reynolds, A. Lobrinus, R.H. Adams, A.S.L. Yu, P. Anand, B.A. Imhof, S. Nourshargh- 2007-Expression and function of junctional adhesion molecule-C in myelinated peripheral nerves- Science 318: (#5855, 11/30) 1472-1475

This article reviews some interesting features of the evolution of human **skin**.

Jablonski N.G- 2010-The naked truth- Scientific American 302: (#2, Feb) 42-49

Even single-celled **bacteria** often show aspects of traits needed by multicellular organisms. For instance their binding to surfaces alters their growth rates, group together to form large **social aggregates** or share items, and some species can communicate to members of a population of bacteria through a system called **quorum sensing**:

Aoki S.K., R. Pamma, A.D. Hernday, J.E. Bickham, B.A. Braaten, D.A. Low- 2005- Contact-dependent inhibition of growth in *Escherichia coli*- Science 309: (8/19) 1245-1248

Kolodkin-Gal I., R. Hazan, A. Gaathon, S. Carmeli, H. Engelberg-Kulka- 2007-A linear pentapeptide is a quorum-sensing factor required for mazEF-mediated cell death in *Escherichia coli*- Science 318: (#5850, 10/26) 652-655

Kolter R- 2007-Deadly priming- Science 318: (#5850, 10/26) 578-579

Marx C.J- 2009-Getting in touch with your friends- Science 324: (#5931, 5/29) 1150-1151

Nudleman E., D. Wall, D. Kaiser- 2005-Cell-to-cell transfer of bacterial enter membrane lipoproteins- Science 309: (7/1) 125-127

Velicer G.J., K.L. Stredwick- 2002-Experimental social evolution with *Myxococcus xanthus*- Antonie van Leeuwenhoek 81: 155-164

White D- 2000-Adaptive and developmental changes- Chapter 18, pgs. 434-533, in, The Physiology and Biochemistry of Prokaryotes. 2nd edition. Oxford University Press, N.Y.

BIO 107   2010
Day 6, Lecture 14, Title: Digestion
Text Readings: Campbell et al. (2008). Chapter 41.

Topics to cover:

      Digestion
      Diffusion as a major factor
      Where to do it: Compartmentalization
      Some non-animal examples
      Animals
            Mammalian Digestive system
            Some variants

Digestion
      Break up of organic matter, to components such as monomers, then
            absorption of the components for use.
      Distinct from: cellular respiration (no ATP production...),
                 excretion (removes wastes made by body).
                 Produces need for elimination of items not digested (feces).

Diffusion as a major factor
      Smaller items diffuse faster, hard to take polymers up across membranes...
      So conversion to smaller sized units makes it easier for molecules to get to cell surfaces.
      Review digestion of each of the major classes of organic matter. Note enzymes for each.
      fig. 27.35, digestive enzymes (Gould etal. 1996)
            Polynucleic acids to nucleotides: via DNAase, RNAase, nucleases
            proteins to amino acids: via proteases
            polysaccharides to monosaccharides: via amylases
            conversion to monomers produces large concentration gradient
                 clearly this enhances the rate of diffusion to cell sufaces.
      Less time to diffuse across shorter distance so
            break up large oil droplets into small ones: via emulsifiers.
      Also break up fats into glycerol and fatty acids: via lipases.

Where to do it: Compartmentalization
      Can not use these digestive enzymes in cytosol, need to avoid self-digestion!
            Could put them in a cellular compartment such as lysozyme/ vacuole.
            So can do intracellular digestion. Most eukaryotic cells do this...
      May also secrete enzymes that would leave cell: Extracellular digestion.
            May leave organism: Extraorganismal digestion.
            Or use a specialized digestive compartment: Intraorganismal digestion.
      Across species compartments. Rabbits eat their feces after microbes have lived in them...

Some non-animal examples
Bacteria option: absorptive
       Fig. 27.2   Rod shaped prokaryotes. Stay small, absorb small things
       Extracellular and extraorganismal digestion, secrete digestive enzymes.
       The products of digestion diffuse to cell surface.
       At cell surface there must be membrane transport systems to take items up.

Fungal option: absorptive
       Fig. 31.2, Fungal mycelium
       Fungal body is not small, but it is thin, giving surface areas to absorb resulting monomers.
       Makes things small by extracellular and extraorganismal digestion.

Carnivorous Plants option: absorptive
       24372_Nepenthes_alata.jpg   pitcher plant
       Extracellular digestion. Secretes digestive enzymes into a pitcher.
       Is the pitcher extra organismal, or a specialized body compartment...??
       Since they are photosynthetic, gets mainly minerals, but some OM as well...
       Items must diffuse to cell surfaces for uptake.

Protist option: absorptive and phagocytotic varies by species
       Consider Paramecium. It eats things like bacteria.
       Fig. 41.10, (Campbell and Reece, 2002) *Paramecium* digestion
       Eat medium sized things, taken into food vacuole.
              phagocytosis results in intracellular and intraorganismal digestion
              secrete digestive enzymes into food vacuoles, via endosomal system
              absorb small things into cytosol across vacuole membrane
       Note that some animal cells also do phagocytosis: relate to animals, sponges, hydra?

Animals
       Digestion in them is very diverse, some lack a digestive tract entirely.
       Hydra
              Fig. 41.11, (Campbell and Reece, 2002) Hydra, GV cavity
              Fig. 41.8, hydra digestion
              Body thin, high surface area, good for diffusion of items to cell surfaces.
              Note gastrovascular cavity:
                     an intraorganismal cavity in which does extracellular digestion.
              Phagocytosis done as well, so some intracellular digestion, just like in paramecium.
              Problems of a G.V. cavity, mouth is annus.
       Complete digestive tract (also called alimentary canal...), several options exist.
              Fig. 41.9, alimentary canal options
              an intraorganismal, but extracellular approach,
                     with specialization along its length.
              Can do different functions at different points along the digestive tract.

Mammalian Digestive system

Mammalian organs in the digestive tract in order are....?

Fig. 41.10, human digestive tract

mouth: salivary amylase, makes maltose. Adds moisture to food.

Does masication via teeth and chewing.

(?Earthworms lack teeth, what does the masication in their digestive tract?)

esophagus: smooth muscles used to drive peristalsis of a packet of food.

note sphincters at both ends of the stomach.

Thus you can stand on your head and not have lunch come out your nose!

stomach: grinding action via smooth muscles, secretes HCl acid,

Fig. 41.12, stomach, gastric juice secretion

and secretes proteases,

and secretes mucus for protection of the stomach lining.

pepsinogen becomes converted to pepsin.

It is an auto-protease (cleaves itself) but only in acid.

How does this help it avoid digesting the cell that makes it?

Fig. 40.05, stomach lining (Campbell and Reece, 2005)

Note high surface area in infolded glands gives places for cells to do

secretion, need for mucus to avoid self digestion

Fig. 41.19, stomach, duodenum, liver, gall, pancreas (Campbell and Reece, 2005)

The stomach is an exocrine gland.

exocrine glands: secrete items out of body.

So sweat glands are exocrine glands.

The digestive tract ultimately exits the body

so glands leading to it are exocrine.

Note that stomach is also an endocrine gland,

as are pancreas and small intestine

small intestine:(duodenum then jejunum, ileum)

Fig. 41.15, small intestine structure

Digestion continues, but so far what organic matter types have been digested?

(In mouth, carbohydrates. In stomach, proteins.)

In intestine do digestion of these and other items.

Also see first major site of absorption of items.

Membrane bound transport and digestive systems held in epithelial cell membranes.

disaccharidases, maltases

enteropeptidase, dipeptidases, etc...

uptake systems

Na^+/item symports, water follows by osmosis,

most water added to food in mouth is recovered here.

entry of hydrophobic vs. hydrophilic items

Secreted by cells in intestine lining are items such as aminopeptidase...

Associated exocrine glands secrete items into the intestine.

    liver produces bile stored in gall bladder, bile acts as emulsifer

        breaks down size of oil droplets

        Bile salts neutralize acids from the stomach.

    pancreas, secretes various proteases and amylases

        carboxypeptidase, trypsin, chymotrypsin

            Each of these have to be activated.

        note, exo vs. endopeptidases

            Exo: cuts off monomer at end of polymer.

            Endo: cuts a bond in middle of polymer.

        pancreatic amylase is distinct from salivary amylase

Review how to activate the digestive enzymes

    stomach: pepsinogen, by change in pH

    intestine a membrane bound enteropeptidase does initial cleavage.

        Note, we can not use a pH shift here...

Fig. 41.20, digestive enzyme activation (Campbell and Reece, 2005)

    It cleaves trypsinogen into trypsin,

        trpsin then converts procarboxypeptidase into carboxypeptidase

        which converts chymotrypsinogen into chymotrypsin

    Note this all starts with an enzyme held on cell surface in intestines.

        So enzymes can not be activated back in the pancreas. A good thing!

    This shows how one of these enzymes is cut to activate it.

    Fig. 27.34, (Gould etal. 1996) chymotrypsinogen.jpg

Digestion in small intestine produces high concentration of monomers.

    These diffuse faster to the cell surfaces for uptake.

Need to maintain this concentration gradient.

    Raising it due to digestion, can lower the concentration near absorbing cells

        by uptake of items there. So need membrane transport systems.

    Also use smooth muscles in intestine to push, mix, move items.

        so not all diffusive movement, but at cell surface it mainly is...

Fig. 41.15, small intestine structure

Have to get items from absorbing cells into blood stream.

    So need capillary beds.

    Note how capillary beds are near high surface area epithelia.

        Note the villi and microvilli.

    Absorption of fats vs. hydrophilic items.

        Which can cross the cell membranes easiest?

        Note the presence of the lymphatic lacteal in the villus.

            Most hydrophobic items enter it.

Can also do endocytosis, and move vesicle to other side of cell to put item in the body.

Fig. 41.13, enzymatic digestion table

Note types of enzymes used for each class of OM digestion. Notes sources.

    review stages and enzymes: proteases, amylases, lipases, nucleases

<u>large intestine (colon)</u>
Fig. 41.x1, (Campbell and Reece, 2002) lining of large intestine
Mainly absorption here, little digestion. And many resident bacteria!
> Last water recovery done, via osmosis so have to take up salts.
>> To do that need transport systems in cells.
> Feces are compacted as dry out. Moved by smooth muscles.
> Note caecum/appendix also has many symbionts living in it.

<u>rectum</u>
Fig. 41.10, human digestive tract
> Another sphincter.
> Elimination is the process of expelling fecal matter.
>> Note that this is not excretion.

Some variants
> herbivores vs. carnivores
> Fig. 41.19, carnivore vs. herbivore digestive tract
>> note change in length of various parts in these two
> Which has the easier food to digest? Where is digestion, where is absorption done?
>> quality of food and difficulty of digestion results in differences
> Fig. 12.8, (Hildebrand and Goslow, 2001) various digestive tracts

> Ruminants, modify the stomach
> Fig. 41.20, ruminant, cow
>> Culture microbes in gut to do digestion for you
>>> especially for digestion of cellulose, via cellulase
>> Animals have no gene for cellulase, so can not eat wood.
>>> So all the wood eating animals use microbes to do this digestion for them.
> So often see symbionts in animals.
>> Fig. 41.x2, (Campbell and Reece, 2002) termites and trichonympha
>> bacteria living in phagosomes in protist, there they digest cellulose
>> protist live in termites, bacteria live in the protist
>> termites swallow wood chips, protists phagocytose them and deliver them to
>>> phagosomes where bacteria digest the cellulose
>> So here, digestion is achieved across several organisms to the benefit of a
>>> community.

In all cases digestion involves the breakdown of complex organic matter to smaller sized components, then must absorb these components. This has consequences for regulation of ion and water content, circulation, etc....

Objectives:

Be able to define digestion. Compare how and where digestion is carried out by bacteria, unicelled protists, fungi, and animals. In animals, how does digestion differ in an animal with a gastrovascular cavity (i.e. hydra) versus in an animal with a complete digestive tract (i.e. humans). How does digestion assist these organisms to over come problems related to diffusion? Where must the items diffuse to and what must happen to them once they get there? In what ways (if any) do these organisms attempt to alter things so as enhance the rates of diffusion of desired items to themselves?

What types of organic matter are absorbed, and what fundamentally limits the rates of absorption? What is the state of the organic matter when it is first consumed, and how is it altered before it is absorbed? Be able to describe the major groups of enzymes involved with digestion of each of the major types of organic matter, and know their origins in the mammalian system.

Know the major organs and their functions in the mammalian digestive tract. What are the specializations of each? Which are exocrine glands and what are the functions of items they secrete? Be able to describe the major functions of each organ in terms of how major types of organic matter consumed is prepared for absorption. How would absorption of a hydrophilic item most likely differ from that of a hydrophobic item?

In what ways does diet correlate with characteristics of a species' digestive tract? For example, how might a herbivore's digestive tract be expected to differ to from that of a carnivore's?

When an organism makes digestive enzymes how does it avoid digesting itself? What are several options an organism has which allow it to avoid this problem? Be able to describe uses of compartments in this regard, either within one cell, or within one organism. Is digestion ever carried out across several organisms?

For review, see self-quiz questions #4-8 of chapter 41.

Needed overheads and items:

fig. 27.35, digestive enzymes (Gould etal. 1996) digestion.jpg
Fig. 27.2, Rod shaped prokaryotes
Fig. 31.2, Fungal mycelium
24372_Nepenthes_alata.jpg   pitcher plant
Fig. 41.10, (Campbell and Reece, 2002) *Paramecium* digestion
Fig. 41.11, (Campbell and Reece, 2002) Hydra, GV cavity
Fig. 41.8, hydra digestion
Fig. 41.9, alimentary canal options
Fig. 41.10, human digestive tract
Fig. 41.12, stomach, gastric juice secretion
Fig. 40.05, stomach lining (Campbell and Reece, 2005)
Fig. 41.19, stomach, duodenum, liver, gall, pancreas (Campbell and Reece, 2005)
Fig. 41.15, small intestine structure
Fig. 41.20, digestive enzyme activation (Campbell and Reece, 2005)
Fig. 27.34, (Gould etal. 1996) chymotrypsinogen.jpg
Fig. 41.15, small intestine structure
Fig. 41.13, digestive enzymes, sources and uses
Fig. 41.x1,(Campbell and Reece, 2002)   lining of large intestine
Fig. 41.10, human digestive tract
Fig. 41.19, carnivore vs. herbivore digestive tract
Fig. 12.8, (Hildebrand and Goslow, 2001) various digestive tracts
Fig. 41.20, ruminant, cow
Fig. 41.x2,(Campbell and Reece, 2002)   termites and trichonympha

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Animal nutrition. Chapter 41. Pages 875-897. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-Biology, 7th edition, Figures 40.05, 41.19, 41.20. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-Biology, 6th edition, Figures 41.10, 41.11, 41.x2, 41.x1. Benjamin Cummings Press. San Francisco, CA.

Gould J.L., W.T. Keeton- 1996-Biological Science. 6th edition. Figures 27.34 and 27.35. W.W. Norton & Co. N.Y., N.Y.

Hildebrand M., G. Goslow- 2001- Analysis of Vertebrate Structure. 5th edition. Fig. 12.8. John Wiley & Sons, Inc. N.Y., N.Y.

Related issues:

When an animal fails to digest the cells it eats this sometimes can result in the evolution of a **symbiotic relationship**. Here is one example:

> McNeil P.L., P.J. McAuley- 1984-Lysosomes fuse with one half of alga-bearing phagosomes during the reestablishment of the european green hydra symbiosis- Experimental Zoology 230: (#3) 377-386

In many cases, there is digestion done in **internal cellular compartments**. Here is an article on an aspect of intracellular digestion:

> Klionsky D.J- 1997-Protein transport from the cytoplasm into the vacuole- Journal of Membrane Biology 157: 105-115

The **epithelial lining** of the digestive tract is under great stress. The rate at which cells in it turn over is rather high:

> Cliffe L.J., N.E. Humphreys, T.E. Lane, C.S. Patten, C. Booth, R.K. Grencis- 2005- Accelerated intestine epithelial cell turnover: A new mechanism of parasite expulsion- Science 308: (6/3) 1463-1465

And there are studies of how **human digestion** and feeding has changed during our **evolution**:

> Leonard W.R- 2002-Food for thought. Dietary change was a driving force in human evolution- Scientific American 287: (#6) 106-115

Of course, the **microbes** that live in our gut can influence our digestion, and hour health.

> Harmon K- 2010-Genetics in the gut- Scientific American 302: (#5, May) 22-24

How did digestion of various items evolve? Here is an article dealing with the evolution in humans of the ability to digest various types of **starch**.

> Cohen J- 2007-A little gene xeroxing goes a long way- Science 317: (#5844, 9/14) 1483

There is much **variation in digestive tracts**. Some species have males who **lack digestive tracts** entirely:

> Pennisi E- 2007-Whale worm sperm factories- Science 315: (1/26) 457

For more on **insect** digestive tracts see:

> Wigglesworth V.B- 1984-Digestion- Chapter 4, pgs. 54-68, in <u>Insect Physiology</u>. Chapman and Hall Publishers, London.

Translucent marine animals try to hide, but food in their gut is often not translucent. So they often enhance their ability to hide by surrounding their digestive tract with reflective surfaces:

> Johnsen S- 2000-Transparent animals- Scientific American 282: (#2, Feb) 80-89

To learn more about **fungal** digestion patterns see:

> Griffin D.H- 1994-Nutrient acquisition: Digestion and transport- Chapter 6, pgs. 158-194, in <u>Fungal Physiology</u>- 2nd edition. Wiley-Liss Press, N.Y.

Digestion as done by the various **carnivorous pitcher plants** is another system:

> Heslop-Harrison Y- 1978-Carnivorous plants- Scientific American 238: (#2) 104-115
> Owen T.P.jr, K.A. Lennon- 1999-Structure and development of the pitchers from the carnivorous plant *Nepenthes alata* (Nepenthaceae)- American Journal of Botany 86: (#10) 1382-1390

Some feeding problems encountered by **herbivores** and what plants do to produce these problems is noted in the following article:

> Sanson G- 2006-The biomechanics of browsing and grazing- American Journal of Botany 93: (#10) 1531-1545

This study examines how a **proenzyme** found in humans can be altered to take on its active state.

> Wolan D.W., J.A. Zorn, D.C. Gray, J.A. Wells- 2009-Small-molecule activators of a proenzyme- Science 326: (#5954, 11/6) 853-858

Studies have found that altering our caloric intake can influence our **lifespan**.

> Fontana L., L. Partridge, V.D. Longo- 2010-Extending healthy life span - From yeast to humans- Science 328: (#5976, 4/16) 321-326

BIO 107   2010
Day 7, Lecture 15, Title: Transport I, Animal Circulation
Text Readings: Campbell et al. (2008), pgs. 898-915.

Topics to cover:

> **Hepatic Portal System**
> **Human Circulation**
>> **Heart Parts**
>> **Control of heartbeat**
>> **Capillary beds and fluid recovery**
> **Other animal systems**
> **Human fetal circulatory changes**
> **Summary**

We left off with the mammalian digestive system, with digested and absorbed items put into capillaries of the small intestine. Continue from there.

Hepatic Portal System
> Where does the food first go from the digestive tract?
>> Fig. 18.21-22, (Marieb et al. 1997) hepatic_portal.jpg
> What is a portal system?
>> hepatic means? (liver)
>> note differences between portal vein vs. hepatic vein
>>> portal vein from small intestine to liver
>>>> connects two capillary beds
>>> hepatic vein goes from liver to posterior vena cava.
>> Why have this portal system? Role of liver in regulating blood contents.

Human Circulation
> Blood   Recall this is a type of connective tissue.
>> So like all connective tissues it has an extracellular matrix,
>>> just not cross linked until there is need for clotting.
>> Putting proteins into RBC rather than blood serum. Why?
>>> If put hemoglobin in blood serum what happens to blood viscosity?
>> Fig. 42.17, compostion of mammalian blood
>> Many cell types, and plasma proteins, from many sources.
> human circulation as an example of mammalian circulation.
> Fig. 42.6, mammalian circulation
>> review sequence of blood flow shown in this figure.
>> Note the pulminary loop versus the systematic loop.
>> Note vessels with oxygenated versus those with deoxygenated blood.

Heart Parts
> Fig. 42.7, human heart

Note parts.

Atria and ventricles. Which ventricle has thicker muscles? Why?

Vena cava, aorta, pulminary veins, note the two types of valves in heart.

Note that in veins have one-way valves to prevent backflow

        Fig. 42.14, flow in veins

        Demonstrate role of valves in veins of the forearm.

        What does this imply about the blood pressure in these veins?

Control of heartbeat

Want a regular sequence, so need to control events well.

        Cardiac muscle cells are well connected cytosolically.

        So action potential travels between them well.

            An example of action potential transmission in non-nervous tissue.

        Fig. 17.4, (Marieb et al. 1997) heart_muscles.jpg

            note how atrial bundles are separate from ventricle bundles.

            so an action potential given to atrial bundles gets to all of them well,

                but does not go to ventricles immediately.

            So atria contract together and first.

        Fig. 42.9, SA node and AV node, heart control

        Note two nodes involved in regulation of heartbeat.

            Sino-Atrial vs. Atrio-Ventricular node, these are made of cardiac tissue.

            SA node also sends signal to left atrium, from there spreads to other atrium

            AV node delays the signal a bit before sending signal to ventricle.

        Also passes signal down bundle of His (i.e. branch bundles) to base of

                ventricle. Why there?

        Bundles and fibers are cardiac muscle tissue, but carry action potentials

            so not all action potential transmission is via nervous tissue.

?Aside. Why pump blood down from atria to ventricle, and then back up out of ventricle?

        Why not have aortae exit from base of heart rather than the apex?

Capillary beds and fluid recovery

Note sphincters, not enough blood to fill entire system, so close off or reduce flow to part

        Fig. 42.15, sphincters in capillary bed

Ways to promote diffusion:

Fig. 42.11, pressure and velocity drop, capillary bed

        Slow blood flow (note change in volume leads to change in veolcity)

            as go into capillaries the total volume increases resulting in slower velocity

            slower blood flow gives more time for exchange.

            Note that volume is proportional to cross sectional area.

                Do not confuse surface area and cross sectional area!

Increase surface area over which to exchange items.
 	Note distinction of surface area and cross sectional area..
 	Capillaries are much higher in surface area than an artery.
Make any barriers thinner,
 	So capillaries are 1 cell layer thick, and are close to cells to be supported
Increase the concentration gradient
 	If blood sites static in capillaries eventually exchange will halt
 	 	because concentration in blood will equal that in surroundings.
 	So keep the blood flowing.
 	Also use transport systems in surrounding cells, and cells of capillaries.
Remember that near to cells most of the movement of items is diffusional.
 	Until it gets to the cell membrane. Then need transport systems.
What happens with water as move through capillary?
 	Fig. 42.16, water loss at capillary bed
 	At arteriole end see: water loss
 	 	Due to high blood pressure that exceeds the osmotic effect of solutes
 	 	 	So a water potential gradient here.
 	At venule end see: water recovery.
 	 	Resistance of the capillaries to blood flow is high.
 	 	 	So lose pressure as move through the bed.
 	 	 	Drop in pressure alters the water potential gradient.
 	 	At venule end osmotic factor exceeds pressure one,
 	 	 	and water potential gradient is reversed relative to arteriole end.
 	Fig. 42.14, water loss at capillary bed (Campbell and Reece, 2005)
 	Recall that water flows due to pressure and concentration gradients.
 	 	water potential, pressure potential, solute potential, changes across bed
 	Not all water is recovered, so use lymphatic system to help get excess.
 	 	Fig. 43.7, human lymphatic system
 	 	 	note how fluid is returned to blood, thoracic duct....
 	 	Fig. 41.15, small intestine
 	 	 	note lymph duct (lacteal) in villus
 	 	 	so fats can get around going through the liver... consequences?
 	 	If lymph vessels are cut fluid can accumulate at extremities.
So we are doing bulk flow to get within diffusional range of local cells.
 	At cellular level still using diffusion to get up to surfaces of cells.
 	At cell membrane still need transport systems to promote exchange...

Other animal systems

Many animals avoid any need for a circulatory system by being small and thin.

Cnidarians, such as jellyfish.

    Fig. 42.2, cnidarian internal transport

    uses gastrovascular cavity.

        Gastro: digestive. Vascular: circulatory.

        Note the radial and circular canals.

Some animals have open circulatory systems.

    open vs. closed circulatory systems

        Fig. 42.3, open vs. closed circulation

    Contrast insect vs. earthworm

        insects get away with this as do not move gases in their blood.

        implications for ability to do gas delivery in the blood for these two animals

fish and amphibians, reptiles all have closed circulatory systems, as do we.

Fig. 42.4, fish circulation

Fig. 42.5, double circulation systems

Some have the ability to send deoxygenated blood to skin for gas exchange

        this occurs in amphibians, and in some reptiles

    This figure (42.5) gets frog a bit wrong.

        blood from pulmocutaneous circuit that returns from

            -lungs comes back via pulmonary vein to left atrium

            -skin comes back via vena cava to right atrium

        Consider how this makes sense of the three chambered heart.

        The figure in the text suggests oxygenated blood always returns to the

            left atrium, which is just not correct.

    This other figure gives another view

        fig. 47.4, amphibian circulation (Brooker et al., 2008)

note extra loop in reptilian heart, allows skin gas exchange to some extent in some species

        Fig. 42.5, double circulation systems

        turtles in cloaca, crocodiles in roof of mouth

    They still have lungs, so still do gas exchange in their lungs.

        This is just another option for which a three-chambered heart works well.

Consider fish circulatory system.

    Two capillary beds in series. How is this similar to the heptatic portal system?

        What does this do to the pressure in the circulatory system?

        Does the blood flow faster or slower than in mammls?

Human fetal circulatory changes
        Our circulatory pattern changes upon birth.

        Fig. 18.24, (Marieb et al. 1997) fetal_circulation.jpg

                review structures:

                ductous veinous: in liver, from umbilical vein to posterior vena cava

                foramen ovale: in heart, form right to left atria

                ductous arteriosus: between the two aortae, systemic and pulmonary

                umbilical vein and artery: from body to placenta and back to liver

        Notice how this results in the systematic loop being the one that picks up oxygen?

                compare this to reptilian system with part of systematic loop doing gas exchange?

        If go back earlier in development the fetal heart also differs.

                Fig. 17.16, (Marieb et al. 1997) fetal_heart.jpg

                early on is just two chambered

        So this works as fetus is not jogging??...

                    would early fetal blood pressure be high or low?

                    heart beat rate fast or slow?

Summary
So point is to generate bulk flow, to move food and wastes, but also for gas exchange.

        Depending on gas exchange system may see various circulatory types...

        If stay small can avoid need for a circulatory system...

Move items within range of diffusion. So at local cellular level is all goes back to diffusion.

Later will view another means of generating bulk flow in a non-animal system.... So this is not the only means to carry out circulation of items in a large organsim....

Objectives:

What is the hepatic portal system, where does it occur, and what role does it play? What is a "portal system"? What part of a fish's circulatory system is a portal system?

Be able to trace the flow of blood through the mammalian heart out to the rest of the body and back again, identifying the major chambers of the heart and immediate veins and arteries connected to it (see figures 42.6 and 42.7 for the level of structures to consider here). How is the heart beat sequence regulated? Be able to describe the role of the nodes.

How do veins differ from arteries? What changes happen to blood pressure and velocity of flow as blood travels away from and back to the heart? Given the limits of diffusion how close must a cell be to a capillary bed?

Blood flow in circulatory beds and accompanying fluid loss is an important issue. What causes the fluid loss? How is fluid that is lost from the blood recovered? Are all capillary beds always open, if not how is the flow through them altered?

What are ways in which blood flow in the human fetus changes at birth to give the adult pattern of circulation? What structures are involved in these changes and what are the reasons for these structures in the human fetus in terms of the flow of blood?

Fish have a two chambered heart. What is the consequence of this on their blood pressure? Frogs breath through either their lungs or their skin, in what way does a three chambered heart make sense in this case? What would allow an animal to have an open circulatory system, and what traits might force an animal to have a closed circulatory system? What are examples of animals for each of these two types of circulation? What is an example of an animal that lacks an internal circulatory system and how does it manage to live without one?

Be able to describe the functional purposes of a circulatory system, and consider the types of energy transductions an organism must carry out to make it work.

For review see self-quiz questions #2 and 3 of chapter 42.

Needed overheads and items:

Fig. 18.21-22, (Marieb et al. 1997) hepatic_portal.jpg
Fig. 42.17, compostion of mammalian blood
Fig. 42.6, mammalian circulation
Fig. 42.7, human heart
Fig. 42.14, flow in veins
Fig. 17.4, (Marieb et al. 1997) heart_muscles.jpg
Fig. 42.9, SA node and AV node, heart control
Fig. 42.15, sphincters in capillary bed
Fig. 42.11, pressure and velocity drop, cap. bed
Fig. 42.16, water loss at capillary bed
Fig. 42.14, water loss at capillary bed (Campbell and Reece, 2005)
Fig. 43.7, human lymphatic system
Fig. 41.15, small intestine
Fig. 42.2, cnidarian internal transport
Fig. 42.3, open vs. closed circulation
Fig. 42.4, fish circulation
Fig. 42.5, double circulation
Fig. 47.4, amphibian circulation (Brooker et al., 2008)
Fig. 42.5, double circulation
Fig. 18.24, (Marieb et al. 1997) fetal_circulation.jpg
Fig. 17.16, (Marieb et al. 1997) fetal_heart.jpg

Handout:

Fig. 18.24, (Marieb et al. 1997) fetal_circulation.jpg
Fig. 47.4, amphibian circulation (Brooker et al., 2008)

References:

Brooker R.J., E.P. Widmaier, L.E. Graham, P.D. Stiling- 2008-<u>Biology</u>. Fig. 47.4.
    McGraw-Hill Inc. N.Y., N.Y.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky,
    R.B.Jackson- 2008-Circulation and gas exchange. Chapter 42. Pages 898-915.
    <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition, Figures 42.14. Benjamin Cummings
    Press. San Francisco, CA.

Marieb E.N., J. Mallatt- 1997-<u>Human Anatomy</u>. 2nd edition. Fig. 17.4, 17.16, 18.24,
    18.22, 18.24. Benjamin/Cummings Press. Menlo Park, CA.

Related issues:

They used to think that cells of the heart just did not get replaced over time. But here is a study that documents slow but real **replacement of cardiac muscle cells**.

> Bergmann O., R.D., Bhardwaj, S. Bernard, S. Zdunek, F. Barnabé-Heider, S. Walsh, J. Zupicich, K. Alkass, B.A. Buchholz, H. Druid, S. Jovinge, J. Fisén- 2009-Evidence for cardiomyocyte renewal in humans- Science 324: (#5923, 4/3) 98-102
>
> Murry C.E., R.T. Lee- 2009-Turnover after the fallout- Science 324: (#5923, 4/3) 47-48

Obviously there is much work on damage that hearts suffer, and with the repair of **damaged heart** tissues or finding ways to replace hearts:

> Cohen S., J. Leor- 2004-Rebuilding broken hearts- Scientific American 291: (#5) 44-51
>
> Ditlea S- 2002-The trials of an artificial heart- Scientific American 287: (#1) 60-69
>
> Hayakawa M., S. Sugiyamma, K. Hattori, M. Takasawa, T. Ozawa- 1993-Age-associated damage in mitochondrial DNA in human hearts- Molecular and Cellular Biochemistry 119: 95-103
>
> Jia T., E.G. Pamer- 2009-Dispensable but not irrelevant- Science 325: (#5940, 7/31) 549-550
>
> van Rooij, E.L.B. Sutherland, X. Qi, J.A. Richardson, J. Hill, E.N. Olson- 2007-Control of stress-dependent cardiac growth and gene expression by a microRNA- Science 316: (4/27) 575-579

New ways to use **cardiac muscle cells** are being found. Including their use in making micropumps.

> Hornyak T- 2007-Microchips with heart- Scientific American 296: (#5, May) 32-33

Heart development is also of interest. Especially the combination of gene expression patterns that must be be used to grow a heart. Others have studied the cells that lead to the cell types found in heart tissues:

> Chien K.R., I.J. Domlan, K.K. Parker- 2008-Cardiogenesis and the complex biology of regenerative cardiovascular medicine- Science 322: (#5907, 12/5) 1494-1497
>
> Couzin J- 2006-Teams identify cardiac "stem cell"- Science 314: (11/24) 1225
>
> Domian I.J., M. Chiravuri, P. van der Meer, A.W. Feinberg, X. Shi, Y. Shao, S.M. Wu, K.K. Parker, K.R. Chien- 2009-Generation of functional ventricular heart muscle from mouse ventricular progenitor cells- Science 326: (#5951, 10/16) 426-429
>
> Olson E.N- 2006-Gene regulatory networks in the evolution and development of the heart- Science 313: (9/29) 1922-1927

Here is a nice article on **reptilian circulation**.

Axelsson M., C.E. Franklin, C.O. Löfman, S. Nilsson, G.C. Grigg- 1996-Dynamic anatomical study of cardiac shunting in crocodiles using high-resolution angioscopy- Journal of Experimental Biology 199: 359-365

Blood vessel formation, **angiogenesis**, how it is controlled is of interest. For instance **cancerous tumors** must form new blood vessels.

Benedito R., R.H. Adams- 2009-Aorta's cardinal secret- Science 326: (#5950, 10/9) 242-243

Bonauer A., G. Carmona, M. Iwasaki, M. Mione, M. Koyanagi, A. Fischer, J. Burchfield, H. Fox, C. Doebele, K. Ohtani, E. Charakis, M. Potente, M. Tiwa, C. Urbrich, A.M. Zeiber, S. Dimmeler- 2009-MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice- Science 324: (#5935, 6/26) 1710-1713

Herbert S.P., J. Huisken, T.N. Kim, M.E. Feldman, B.T. Houseman, R.A. Wang, K.M. Shokat, K.Y.R. Stainier- 2009-Arterial-venous segregation by selective cell sprouting: An alternative mode of blood vessel formation- Science 326: (#5950, 10/9) 294-298

Jain R.K., P.F. Carmeliet- 2001-Vessels of death or life- Scientific American 285: (#6) 38-45

Olive K.P., M.A. Jacobetz, C.J. Davidson, A. Gopinathan, D. McIntyre, D. Honess, B. Modhu, M.A. Goldgraben, M.E. Coldwell, D. Allard, K.K. Frese, G. DeNicola, C Feig, C. Combs, S.P. Winter, H. Ireland-Zecchini, S Reichelt, W.J. Howat, A Chang, M. Dhara, L. Wang, F. Ruckert, R. Grützmann, C. pilarsky, K. Izeradjene, S.R. Hingorani, P. Huang, S.E. Davies, W. Plunkett, M. Egorin, R.H. Hruban, N. Whitebread, K. McGovern, J. Adams, C. Iacobuzio-Donahue, J. Griffiths, D.A. Tuveson- 2009-Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer- Science 324: (#5933, 6/12) 1457-1461

Olson P., D. Hanahan- 2009-Breaching the cancer fortress- Science 324: (#5933, 6/12) 1400-1401

What the blood carries is also of interest, for example **monocytes** are part of the immune system and they interact with the cells that make up the blood vessel walls. **Platelets** are made by cells in the liver and put into the bloodstream. Also nitrous oxide, and other **reactive oxygen species**, have been reported to act as signals in vasodilation.

Auffray C., D. Fogg, M. Garfa, G. Elain, O. Join-Lambert, S. Kayal, S. Samachki, A. Cumano, G. Lauvau, F. Geissmann- 2007-Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior- Science 317: (#5838, 8/3) 666-670

Geddis A.E., K, Kaushansky- 2007-The root of platelet production- Science 317: (#5845, 9/21) 1689-1690

Hartzell H.C- 2007-The stress of relaxation- Science 317: (#5843, 9/7) 1331-1332

How long can you preserve mammalian organs without any circulatory support? In addition to cooling the organs, changing their oxygen content and redox state seems to have an influence, and studies of this may one day lead to true **suspended animation** of at least organs if not entire organisms:

Roth M.B., T. Nystal- 2005-Buying time in suspended animation- Scientific American 292: (#6, June) 48-55

The **blood/brain barrier** is a region with very tight sets of capillaries in the circulatory system.

Lammert E- 2008-Brain WNT for blood vessels- Science 322: (#5905, 11/21) 1195-1196

Stenman J.M., J. Rajagopal, T.J. Carroll, M. Ishibashi, J. McMahon, A.P. McMahon- 2008-Canonical Wnt signaling regulates organ-specific assembly and differentiation of CNS vasculature- Science 322: (#5905, 11/21) 1247-1250

Here is an article that relates to one of the **clotting factors** of blood.

Zhang X., K. Halveorsen, C-Z. Zhang, W.P. Wong, T.A. Springer- 2009-Mechanoenzymatic cleavage of the ultralarge vascular protein von Willebrand factor- Science 324: (#5932, 6/5) 1330-1334

BIO 107   2010
Day 7, Lecture 16, Title: Gas Exchange
Text Readings: Campbell et al. (2008), pgs. 776-779, 915-927.

Topics to cover:

> **Gas exchange issues**
> **Plant system**
> **Fungal and bacterial system**
> **Animal systems**
>> **Counter current exchange system**
>> **Partial pressures**
>> **Hemoglobin and saturation curves**

Gas exchange issues

For most large multicelled organisms diffusion at the surface skin is often insufficient.
> So need distinct specialized regions of the body where gas exchange occurs.

Many questions concerning gas exchange:
> Through what medium are the gases exchanged? Gas or water?
>> In either case ends up entering cells in water. Moist local surfaces.
> What distances possible for diffusion in each?
>> In air about centimeters, in water about millimeters...
> Need O_2 for? CO_2 made by? Are any other gases taken in or released?
> Are there special carrier systems?
> Does the gas move via a circulatory system or not?
>> What happens when it shuts down? Dormancy or death?
> How is this system regulated?

Plant system
> Plant roots need oxygen gas. They do cellular respiration.
>> So water logged roots may die.
> Leaves have to take up carbon dioxide.
>> Recall the C4 system of CO_2 movement.
>> A means of internal movement of this gas in plants.
>> Fig. 36.12, diffusion of gases out of a leaf (Campbell and Reece, 2005)
>> Note that O_2 leaves and CO_2 must be taken in.
>>> Also loss of water in gaseous form occurs.
> How do leaves regulate gas exchange?
>> Waxy cuticle limits exchange to somates.
>> Pore in stomates regulated in size.
>> Fig. 36.17, stomatal cell swelling and shrinking
>> Note hyperpolarization of PM, via activation of pump
>>> driving K^+ uptake, leading to osmotic swelling, pore opens.
>> Fig. 36.14, stomata SEM (Campbell and Reece, 2005)

So each stomatal pore can be thought of as analogous to our lips and nose.

Fungal and bacterial system
> Both stay small enough for diffusion, but can have changes in environment
>> So they must be able to tolerate these conditions.
>
> May need to live under anaerobic conditions? Fermentative abilities?
>
> May need option to go dormant?

Animal systems
> Consider a few animals and how their gas exchange needs alters aspects of them
>
> Planaria and Nudibranchs
>> Not the fastest of animals, so moderate to low oxygen gas demands.
>>
>> These animals are thin enough for gas exchange via diffusion across body surface.
>>
>> Fig. 33.10, Planaria, protonephridia
>>
>> Fig. 33.19, (Campbell and Reece, 2002)   nudibranch
>>
>> So no distinct gas exchange system in these animals.
>>> Therefore not all animals have such a system. Consider sponges..
>
> Earthworm
>> Tunnelling takes muscle work, high energy demand, so high oxygen demand.
>>
>> So a need for high rate of oxygen gas uptake, and need gas exchange system.
>>
>> Fig. 42.3, insect vs. earthworm circulation
>>> Note that this also relates to closed circulatory system seen.
>>>> relate circulation to gas exchange systems
>>
>> Fig. 44.12, earthworm, metanephridia
>>
>> respiration via skin, note fluid filled body cavity as well.
>>
>> so must keep the skin moist, poor for controlling water loss?
>>> So earthworms stay underground mainly to prevent water loss.
>>>
>>> Why do they come aboveground during rainstorms?
>
> Insect
>> Very active metabolism, flight, diggers, etc, so also have high oxygen demand.
>>
>> Use tracheal system, air filled tubes with openings on side of body.
>>> Fig. 42.23, insect tracheal systems
>>
>> diffusion in air about 10000 > than in water
>>> So can move gaseous items centimeters by diffusion.
>>>
>>> Note that this is about the upper size of insects...
>>
>> can also pull/push air in and out, tubes can be opened or closed, note sacs.
>>
>> diffusion is done near cell and into and out of cells
>>> All the cells have local moist surfaces. So moves into water medium.
>>
>> note the implications for circulatory system...
>>> do insects need one to carry oxygen?

Counter current exchange system

Fish

  May have bursts of activity, but relatively moderate oxygen demands otherwise.

  But water is not the best source of oxygen gas. So use gills

  Fig. 42.22, fish gills

    oxygen concentration in water is low (10-300 µM level), need to pull it out

    warmer water dissolves less oxygen gas...

  Note the use of a counter current exchange system

  Fig. 42.21, (Campbell and Reece, 2002) countercurrent exch. in fish gills

  maintains concentration gradient along entire length, this promotes diffusion

    note role of circulatory system to maintain concentration gradient from

      blood's side of things.

Human vs. bird Humans lack counter current system, birds have one

  Air breathing can supply more oxygen gas, support a higher metabolic rate

  Humans do this in their lungs, note the

    high surface area, thin exchange surfaces, moist surfaces

    Fig. 42.24, human lungs, alveoli

    and blood flow keeps the concentration gradient present.

    So all exchanges is via diffusion, but no counter-current system here...

  Humans use a diaphragm.

    Fig. 42.25, negative pressure breathing

    diaphragm, dead ended so much stagnant air, thoracic cavity

  Contrast air flow through human lungs to that of birds.

    Fig. 42.26, bird lungs

    note one way flow, more efficient than humans

    due to one way flow of air through lungs vs. blood flow, countercurrent

    Birds can pull oxygen out of the air much better than mammals can.

    So humans die at top of mountains as birds fly by....

Partial pressures

  Fig. 42.27, blood partial pressures and gas exch. (Campbell and Reece, 2005)

  Partial pressure. In terms of one atmosphere what part is due to a gas.

    Typically done in terms of pressure units of mm of Hg

    For oxygen gas = (0.20)(760 mm Hg)

  note the typical partial pressures of oxygen gas

    pulmonary veins (104 mm Hg) versus pulmonary arteries (40 mm Hg)

    systematic arteries (104 mm Hg) versus systematic veins (40 mm Hg)

    Air entering (160 mm Hg) and leaving (120 mm Hg) the lungs.

      (see fig. 42.28 in text for CO_2 partial pressures)

  Due to dead/stagnant air do not get full exchange when breathe

    note how this limits extent of gas exchange achieved

    note venous blood need not lose all oxygen gas

Hemoglobin and saturation curves

Hb saturation curves

Recall the structure of hemoglobin, heme cofactor, oxygen binding site

Fig. 5.21, quaternary structure, hemoglobin

Relate oxygen binding by hemoglobin to concentration of oxygen in air

Fig. 42.29a, hemoglobin dissociation curve

review how to read dissociation curve

Hb's oxygen binding is pH dependent, this is called the Bohr shift.

Consider pH changes in blood with circulation

What alters the pH of the blood? Where is this item made and how?

Hb is in cells

Fig. 42.30, RBC and CO_2 and Hb

Fig. 42.29b, hemoglobin and pH

otherwise blood would be very viscous!

So easier on the heart to pump blood with cells rather than viscous blood serum

Note forms CO_2 takes, some forms are bound by Hb, note effects on pH

RBC

Origins and loss, hundreds of millions lost each day

Role of spleen, to remove dead RBC,

also immune system checks them out...

fetal Hb

Have to pull oxygen from adult hemoglobin in placenta

Fig. 42.UN897, fetal Hb

note K_m of fetal Hb relative to adult Hb, how is this adaptive?

other types of O_2 carriers/binders exist

myoglobin

Found in muscle cells

Fig. 42.30, (Campbell and Reece, 2002) seals

use myoglobin to store oxygen

allows seals to dive for many minutes without breathing

Also in our muscle cells, used for bursts of high oxygen demand periods

Note different affinity for oxygen than Hb

Fig. 21.13, hemoglobin family of genes

Leghemoglobin

part of this made by bacteria, part by plants

Why would bacteria make an oxygen binding protein?

hemocyanin

copper based system.

So lobsters have blue-blood due to this system...

If time:

Note ancient earth was anaerobic, early oxygen gas was very toxic, so needed to bind it up to protect anaerobes. Permian extinction event and drop in oxygen from 30% to 10%!

Objectives:

Plants, fungi, and animals such as planaria, earthworms, insects, fish, birds and humans all must deal with the exchange of gases. Be able to compare how each of them achieves this essential goal. What difference does the medium make through which they attempt to carry out gas exchange?

Be able to describe how plants open and close their stomata to control gas exchange. What gases move through their stomatal pores?

What are the advantages and disadvantages of breathing through skin versus breathing through a specialized gas exchange structure such as a lung, a tracheal opening, a stomatal pore, or a gill? What structural aspects of the alveoli improves its effectiveness for gas exchange? Be able to point to similar aspects in gills and trachea and in leaves.

How does counter current exchange increase the efficiency of gas exchange? What are examples of organisms that have counter current gas exchange systems and others that do not?

Be able to relate the saturation level of hemoglobin in human blood to its location in the circulatory system. Be able to use information in hemoglobin saturation graphs to support such comparisons. What is partial pressure, and how does the partial pressure of oxygen gas change across the circulatory system? How does the exchange of carbon dioxide interact with the exchange of oxygen? Since myoglobin and fetal hemoglobin have greater affinities for oxygen than does the regular adult hemoglobin explain why these other oxygen binding proteins have not replaced adult hemoglobin.

For review see the self-quiz questions #1, 5, 7, 8 and 11 for chapter 42.

Needed overheads and items:

Fig. 36.12, (Campbell and Reece, 2005) diffusion of gases out of a leaf
Fig. 36.17, stomatal cell swelling and shrinking
Fig. 36.14, (Campbell and Reece, 2005) stomates SEM
Fig. 33.10, Planaria,
Fig. 33.19, (Campbell and Reece, 2002)   nudibranchs
Fig. 42.3, insect vs. earthworm circulation
Fig. 44.12, earthworm, metanephridia
Fig. 42.23, insect tracheal systems
Fig. 42.22, fish gills
Fig. 42.21,(Campbell and Reece, 2002)   countercurrent exch. in fish gills
Fig. 42.24, human lungs, alveoli
Fig. 42.25, negative pressure breathing
Fig. 42.26, bird lungs
Fig. 42.27, (Campbell and Reece, 2005) blood partial pressures and gas exch.
Fig. 5.21, quaternary structure, hemoglobin
Fig. 42.29a, hemoglobin dissociation curve
Fig. 42.30, RBC and CO_2 and Hb
Fig. 42.29b, hemoglobin and pH
Fig. 42.UN897, fetal Hb
Fig. 42.30, (Campbell and Reece, 2002) seals
Fig. 21.13, hemoglobin gene family

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Circulation and gas exchange. Chapter 42. Pages 915-927, and pgs. 776-779. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-Biology, 7th edition, Figures 36.12, 36.14, 42.27. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-Biology, 6th edition, Figures 33.19, 42.21, 42.30. Benjamin Cummings Press. San Francisco, CA.

Related issues:

Of course **lungs** have to deal with more than just gas exchange. They also need very active **immune system** cells to resist the growth of pathogens suchs as bacteria and viruses.

Kowalski M.P., A. Dubouix-Bourandy, M. Balmoczi, D.E. Golan, T. Zaidi, Y.S. Coutinho-Siedge, M.P. Gygi, S.P. Gygi, E.A. Wiemer, G.B. Pier- 2007-Host resistance to lung infection mediated by major vault protein in epithelial cells- Science 317: (#5834, 7/6) 130-132

The **heme cofactor** has been shown to have many other roles than just that with hemoglobin. One role is in the circadian rhythm of some species.

Yin L., N. Wu, J.C. Curtin, M. Qatanani, N.R. Szwergold, R.A. Reid, G.W. Waitt, D.J. Parks, K.H. Pearce, G.B. Wisely, M.A. Lazar- 2007-Rev-erbα, a heme sensor that coordinates metabolic and circadian pathways- Science 318: (#5857, 12/14) 1786-1789

Hemoglobin is an oxygen-binding molecule. Various forms of hemoglobins are found in organisms other than animals where they often act as oxygen concentration sensors.

Arredondo-Peter R., M.S. Hargrove, J.F. Moran, G. Sarath, R.V. Klucas- 1998-Plant hemoglobins- Plant Physiology 118: (#4) 1121-1125

Barker D.G., P. Gallusci, V. Lullien, H. Kahn, M. Ghérardi, T. Huguet- 1988- Identification of two groups of leghemoglobin genes in alfalfa (*Medicago sativa*) and a study of their expression during root nodule development- Plant Molecular Biology 11: 761-772

Nio X., R.D. Hill- 1997-Mitochondrial respiration and hemoglobin gene expression in barley aleurone tissue- Plant Physiology 114: 835-840

Sankaran V.G., T.F. Menne, J. Xu, T.E. Akie, G. Lettre, B. van Handel, H.K.A. M. Khola, J.N. Hirschhorn, A.B. Cantor, S.H. orkin- 2008-Human fetal hemoglobin in expression is regulated by the developmental stage-specific repressor *BCL11A*- Science 322: (#5909, 12/19) 1839-1843

Oxygen gas is also known to produce **oxidative stress**. Thus aerobic organisms have to have mechanisms to deal with reactive oxygen.

Semenza G.L- 2007-Life with oxygen- Science 318: (#5847, 10/5) 62-64

With our circulatory system supplying our tissues with **oxygen gas** we tend to take the view that life with oxygen is normal. Yet there are many habitats that lack adequate oxygen and are called **anoxic**. They either vary in oxygen content, or lack it, and life forms must therefore live in the absence of oxygen gas using **anaerobic** means. Plant roots are a good example of this and some soil dwelling organisms such as nematodes, but in our intestines there are also many anaerobic microorganisms.

Anderson L.L., X. Mao, B.A. Scott, C.M. Crowder- 2009-Survival from hypoxia in *C. elegans* by inactivation of amino acyl-tRNA synthetases- Science 323: (#5914, 1/30) 630-635

Crowder C.M- 2009-Ceramides - Friend or foe in hypoxia?- Science 324: (#5925, 4/17) 343-344

Drew M.C- 1997-Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia- Annual Review of Plant Physiology and Plant Molecular Biology 48: 223-250

Eckberg P.B., E.M. Bilk, C.N. Bernstein, E. Purdom, L. Dethlefsen, M. Sargent, S.R. Gill, K.E. Nelson, D.A. Relman- 2005-Diversity of the human-intestinal microbial flora- Science 308: (6/10) 1635-1638

Porterfield D.M., M.E. Musgrave- 1998-The tropic response of plant roots to oxygen: Oxytropism in *Pisum sativum* L.- Planta 206: 1-6

Verslues P.E., E.S. Ober, R.E. Shart- 1998-Root growth and oxygen relations at low water potentials. Impact of oxygen availability in polyethylene glycol solutions- Plant Physiology 116: 1403-1412

Ancient life did not have high concentrations of **oxygen gas** available. Thus there has been shifts in oxygen use in metabolism over the course of **evolution**. For instance 250 million years ago the concentration of oxygen gas in the atmosphere was much higher than today. The shift of oxygen gas to lower concentrations is thought to have influenced dinosaur, bird, and mammal evolution.

Berner R.A., J.M. Vanden-Brooks, P.D. Ward- 2007-Oxygen and evolution- Science 316: (4/27) 557-558

Canfield D.E., S.W. Poulton, G.M. Narbonne- 2007-Late-neoproterozoic deep-ocean oxygenation and the rise of animal life- Science 315: (1/5) 92-95

Kaufman A.J., D.T. Johnston, J. Farquhar, A.L. Masterson, T.W. Lyons, S. Bates, A.D. Anbar, G.L. Arnold, J. Garvin, R. Buick- 2007-Late Archean biospheric oxygenation and atmospheric evolution- Science 317: (#5846, 9/28) 1900-1903

Kerr R.A- 2006-A shot of oxygen to unleash the evolution of animals- Science 314: (12/8) 1529

Oschmann W- 2003-Oxygen in the ocean- in Palaeobiology II. Chapter 4.3.2, pgs. 470-472. D.E.G. Briggs and P.R. Crowther editors. Blackwell Pub. Oxford, U.K.

This study found that **alligators** have **one-way flow** of air through their lungs, just like **birds**.

Farmer C.G., K. Sanders- 2010-Unidirectional air flow in the lungs of alligators- Science 327: (#5963, 1/15) 338-340

BIO 107   2010
Lecture 17, Title: Plant Nutrition
Text Readings: Campbell et al. (2008), Chapter 37.

Topics to cover:

>   **Mineral requirements**
>   **Plants need transport systems to take up essential minerals**
>   **Factors influencing availability of soil nutrients to plants**
>   **Example: Nitrogen as an essential mineral**
>   **When all else fails: Predation or parasitism!**

Mineral requirements
Essential items (other than CO_2, H_2O and O_2 ...)
>   Table 37.1, essential minerals
>   Notice, no organic matter. Autotrophs make their own organic matter.
>   So autotrophic nutrition is mineral-based.
Two broad classes of mineral nutrients, based on amount needed.
Macronutrients
>   needed at > 30 µmoles/g dry soil
>   includes N, K, Ca, Mg, P, S
>   these are the major fertilizers we apply to our crops
Micronutrients
>   needed at < 3 µmole/g dry soil
>   includes Cl, B, Fe, Mn, Zn, Cu, Mo
Determining mineral requirements
>   Use of hydroponics, with defined nutrient solution
>   >   Can remove selected elements and see if needed.
>   (Fig. 37.3, Campbell and Reece, 2002; magnesium deficiencies)
>   Mobile nutrients are moved to where needed, immobile are not.
>   Mg deficiency, mobile nutrient, chlorosis in older tissue as Mg is moved to younger..
>   Versus Fe deficiency, immobile nutrient, chlorosis in younger tissue
>   >   can see younger tissue feed on older, source and sink concept

Plants need transport systems to take up essential minerals
>   Many of these occur at cell membranes, need a transport system for each ion.
>   Fig. 37.2, uptake of nutrients (Campbell and Reece, 2005)
>   Fig. 7.19, cotransport, H^+/sucrose symport system
>   Recall that at membrane surfaces have diffusion up to the membrane.
>   Electrochemical gradient of H^+ created by H^+-ATPase, so costs energy.
>   Uptake by cotransport system allows a local lowering of concentration of mineral.
>   >   This produces a concentration gradient, powering diffusion.
>   How is this similar to uptake systems we saw in the mammalian intestine?

Factors influencing availability of soil nutrients to plants
  Plant growth depends on available nutrients
  (Fig. 7.2, Salisbury and Ross, 1992, root_soil_mining.jpg)
    We can note how plant alters its growth with nutrient availability changes
    High nutrient levels or low levels for P, N, or K alter plant growth
  Plant actively senses the soil and grows accordingly
    So plant has receptors for items in the soil.
  Here will consider several factors in the soil that influence mineral availability

soil composition, what it is made up of can alter its content.
  Different soils are based on different minerals
  Differ also in cation exchange capacity, ability to hold cations on soil negative surfaces
  (Fig. 37.3, soil water and minerals)
  Clay has negative charges, cations stick to it
plant secretions, the plant can secrete items, ions, organic matter, peptides, etc.
  This produces an area around the roots with distinct contents, call this the rhizosphere
  Plant secretes H^+, this displaces cations from soil particles and makes them more available
  (Fig. 5.6, Taiz and Zeiger, 1991, pH_soil_nutrients.jpg)
    note how pH alters availability of various nutrients.
    so soil pH matters
      can be too alkaline or too acidic and solubility of some minerals drop
      bogs, very acidic, so nutrients often limiting
      in alkaline lakes can also see mineral limitations
  How can a plant get a mineral if it will not dissolve well at the soil's pH?
  Plant secretes chelators that bind the mineral and carry it.
  (Fig. 5.10, Taiz and Zeiger, 1991, EGTA_chelator.jpg)
    note how this molecule wraps about Fe
    improves its availability to plant as iron is often not very soluble
    so plant senses Fe in itself, and in soil, only secretes chelators if necessary
      this chelator is organic matter, it is expensive to secrete
soil bacteria alter the nature of the soil
  Rhizosphere
    region of soil that plant secretions and absorption influences
    this also often alters local microbe populations
    bacteria use plant secretions as food, for instance that chelator
    bacteria do metabolism on minerals, alter their availability to plants
soil fungi, also can live in the soil around the plant roots in the rhizosphere
  mycorrhizae are plant/fungal interactions in a symbiosis
  The majority of plants have fungal mycorrhizae.
  Try to grow plants in their absence and often they will not grow well.
  (Fig. 7.6, Salisbury and Ross, 1992, juniper_mycorrhizae.jpg)
    These tree seedlings do much better with mycorrhizae than not.

Two types of mycorrhizae noted.
Ectomycorrhizae, in plant cell walls, but not into space of plant cells.
(Fig. 5.7, Taiz and Zeiger, 1991, ectomycorrhizae.jpg; Fig. 37.12, mycorrhizae)
Endomycorrhizae, often entend into space of plant cells,
      with plant membrane around them. Lots of surface area here.
(Fig. 5.8, Taiz and Zeiger, 1991, endomycorrhizae.jpg; Fig. 37.12, mycorrhizae)
Plant gets nutrients from the fungus. Fungus has a very high surface area in the soil.
Fungus gets OM from plant, such as sugars and amino acids.
If this gets out of balance, the fungus can become a plant pathogen.
      Or the plant can be pathogenic on the fungus.
      so there is natural selection on how each species acts in this symbiosis

Example: Nitrogen as an essential mineral
Nitrogen is often the most limiting mineral in our fields of crops.
      Nitrogen fertilizer is a major expense of agriculture.
Typical forms of inorganic nitrogen in the soil include
      (fig. 37.9, bacteria and nitrogen nutrition)
      N_2, NH_4^+, NO_3^-, NO_2^-
      Note first is gaseous, but its triple bond is too tough to break. Plants can not use it.
      Any of the other three plants can take up
          and use to make the amine group in amino acids and base of nucleic acids.
Factors influencing available soil nitrogen
      Presence of microbes.
      A bacterium wants to use ammonium ion to make its amino acids, and so does a plant
          who gets to take it up depends on many factors.
      Nitrogen can be present in the soil, but locked up in bacteria, and so not available to plants
      Bacteria also alter the form of nitrogen in the soil.
          Note that there is energy in N oxidation, just as in C oxidation.
             So converting N forms allows bacteria to get access to chemical energy
      Processes bacteria do in the soil include
          Ammonification: Organic forms of nitrogen are converted to inorganic forms
             OM nitrogen to ammonium, this can increase the soluble N in soil
             Ammonium ion is very soluble, so can get to plant roots
             but can also wash out of the soil
          Nitrifying bacteria: ammonium to nitrate
             nitrate is also soluble, so that is good
          Denitrifying bacteria: nitrate to N_2
             This releases N to a gaseous form, which leaves the soil
                this lowers the N content of the soil
          Nitrogen fixing bacteria: N_2 to ammonium
             Cyanobacteria, *Rhizobium* sp. etc...
             only prokaryotes do this, no eukaryotes!

Nitrogenase enzyme is sensitive to free oxygen gas,
so this process evolved before O_2 in atmosphere! Ancient!
Plant root nodules are where nitrogen fixing bacteria are encouraged to live
Root nodules
(fig. 37.10a, root nodules)
What the bacterium needs and supplies.
Organic matter from plant, ammonium from bacterium
anaerobic conditions are needed so that nitrogenase can work
but to respire the sugars need oxygen gas to do aerobic respiration to make ATP
The plant helps to supply these contradictory conditions.
Sugars it brings to the nodule via its vascular tissue
Leghemoglobin to bind free O_2, is at 700 μM in cytosol
(Taiz and Zeiger, 1991)
makes nodules look pinkish
globin protein is made by the plant,
heme is made by bacterium
acts as an O_2 buffer
$K_m(O_2)$ 10-20 nM
(human hemoglobin has $K_m(O_2)$ about 126 nM)
Thus free oxygen gas concentration is low,
yet leghemoglobin can supply oxygen gas to mitochondria and bacteria
Ammonium made by bacteria is transported out of nodule to rest of plant via the plant's
vascular tissue
Steps in nodule formation involves detailed interactions between plant and bacterium
(fig. 37.11, soybean root nodules)
Infection thread forms
Bacteriods form in cortical cells in vesicles. Recall endosymbiosis?
(fig. 37.10b, root nodule TEM)
(fig. 37.11, soybean root nodules)
cells divide in pericycle and cortex
Nodule developes in cortex
Vascular connections form
Signals from plant to bacterium and back can activate genes
(fig. 37.13, Campbell and Reece, 2002; signaling for root nodule formation)
flavonoid from plant
bound by NodD in bacterium
this binds DNA and activates nod genes
forms nod enzymes
forms nod factors
nod factors pass back to plant, and by recognizing them the plant alters its growth
This is related to the innate immune system in plants

When all else fails: Predation or parasitism!

    Epipactis sp. (helleborine orchid)

        (Newcomb 1977; pg. 49, Epipactis.jpg)

        (Epipactis helleborine.jpg; Chapman 1997; pg. 113)

        an introduced orchid, feeds on fungi, which feed on pines

        So pine trees do photosynthesis and feed the fungus and get minerals from fungus

        Fungus also gets hormones from this orchid.

        Orchid traps endomycorrhizae and digest them.

            (fig. 37.12, endomycorrhizae)

        So gets organic matter from fungus

        Therefore this orchid need not do any photosynthesis, so needs no chlorophyll

        Thus it can live underground for decades, and when emerges can have white leaves

    Indian Pipe

        (fig. 37.14, Indian pipe)

        also makes no chlorophyll, does no photosynthesis ever!

        has connections to fungi that get food from other plants

    Pitcher plant

        (24379_traps_of_Nepenthes.jpg)

        gets N from traped insects, secreted digestive enzymes

        In this case digest the organic matter to get the N, can also absorb amino acids

            but those are not essential since it can make its own once is has N

        One species in Borneo has traps large enough to catch rats!

    Sundew

        (Fig. 37.14, carnivorous plants)

        Quicktime movie, 37.16-SundewTrapPrey-B (Campbell and Reece, 2005)

        wraps about insects, then secretes digestive enzymes

        Needs a sense of touch, needs ability to change shape of its body

            needs multicellular senses, transduction, response systems.

So plants have nutritional needs, and they have options for obtaining these minerals.

    Grow through soil to it.

    Alter soil state, via pH, or secreted items such as chelators.

    Mutualistic relations, with fungi or bacteri

    Eat a fungus or trap an animal.

    Or just trick humans into caring for you!

Objectives:

What are the differences between macronutrients and micronutrients of plants? Be able to identify a few of each. You should be able to propose several different hypothetical transport mechanisms that plants could use to bring any one type of nutrient into their cells. Where in the process of uptake of items from the soil do diffusion limits matter?

Describe several factors that can alter the availability to a plant of nutrients in the soil. What might the plant attempt to do to improve its access to nutrients? In what ways might plants interact with soil microorganisms to improve the plant's nutrient status and what benefit might the soil microbes get from this association? What is the rhizosphere? Be able to describe fungal interactions with plants.

You should be able to describe the major steps in the process of root nodule formation in plants. What conditions must the plant create to promote nitrogen fixation by the bacterium? What roles do flavonoids and nod factors play in the initiation of root nodule formation?

Instead of getting their essential minerals from inorganic sources what are examples of plants that obtain their minerals from organic sources and how do they manage to do this?

For review, see self-quiz questions #3-9 of chapter 37.

Needed overheads and items:

      Table 37.1, essential minerals
      Fig. 37.3 (Campbell and Reece, 2002) magnesium deficiency
      Fig. 37.2, (Campbell and Reece, 2005) uptake of nutrients
      Fig. 7.19, cotransport, H^+/sucrose symport system
      Fig. 7.2, root_soil_mining.jpg (Salisbury and Ross, 1992)
      Fig. 37.3, cation exchange in soil
      Fig. 5.6, pH_soil_nutrients.jpg (Taiz and Zeiger, 1991)
      Fig. 5.10, EGTA_chelator.jpg (Taiz and Zeiger, 1991)
      Fig. 7.6, juniper_mycorrhizae.jpg (Salisbury and Ross, 1991)
      Fig. 37.12, mycorrhizae
      Fig. 5.7, ectomycorrhizae.jpg (Taiz and Zeiger, 1991)
      Fig. 37.12, mycorrhizae
      Fig. 5.8, endomycorrhizae.jpg (Taiz and Zeiger, 1991)
      Fig. 37.9, nitorgen acquisition
      Fig. 37.10a, root nodules
      Fig. 37.11, soybean root nodule
      Fig. 37.10b, bacteroids
      Fig. 37.11, soybean root nodule
      Fig. 37.13 (Campbell and Reece, 2002)
      Epipactis.jpg (Newcomb 1977; pg. 49)
      Epipactis helleborine.jpg   (Chapman 1997; pg. 113)
      Fig. 37.12, mycorrhizae
      Fig. 37.13, parasitic plants
      24379_traps_of_Nepenthes.jpg
      Fig. 37.14, carnivorous plants
      Quicktime movie, 37.16-SundewTrapPrey-B (Cambell and Reece, 2005)

Handout:
      Fig. 5.6, pH_soil_nutrients.jpg (Taiz and Zeiger, 1991)
      Fig. 37.13 (Campbell and Reece, 2002)

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Soil and plant nutrition. Chapter 37. Pages 785-800. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition, Figure 37.2. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figures 37.3, 37.13. Benjamin Cummings Press. San Francisco, CA.

Chapman W.K- 1997-<u>Orchids of the Northeast. A field guide</u>. Pg. 113. Syracuse University Press. Syracuse, N.Y.

Salisbury F.B., C.W. Ross- 1992-<u>Plant Physiology</u>, 4th edition, Figures 7.2, 7.6. Wadsworth Publishing Co. Belmont, CA.

Taiz L., E. Zeiger- 1991-<u>Plant Physiology</u>. Figures 5.6, 5.7. 5.8. 5.10, and pgs 295-296. Benjamin/Cummings Publishing. Redwood City, CA.

Related issues:

Concerning **mycorrhizae**. How this relationship is formed, what signals are sent, how it benefits the plant are all of interest. Mycorrhizae are known to have effects on ecosystems, especially in terms of the cycling of nutrients. Also some mycorrhizae, while benefiting one species has been known to be pass nutrients from one plant to another, facilitating plant parasiticism.

Anderson R.C., E.S. Menges- 1997-Effects of fire on sandhill herbs: Nutrients, mycorrhizae, and biomass allocation- American Journal of Botany 84: (#7) 938-948

Bidartondo M.I., A.M. Kretzer, E.M. Pine, T.D. Bruns- 2000-High root concentration and uneven ectomycorrhizal diversity near *Sarcodes sanguinea* (Ericaceae): A cheater that stimulates its victums?- American Journal of Botany 87: (#12) 1783-1788

Boerner R.E.J- 1990-Role of mycorrhizal fungus orgin in growth and nutrient uptake by *Geranium robertianum*- American Journal of Botany 77: (#4) 483-489

Drissner D., G. Kunze, N. Callewaert, P. Gehrig, M. Tamaslaukht, T. Boller, G. Felix, N. Amrhein, M. Bucher- 2007-Lyso-phosphatidyl choline is a signal in the arbuscular mycorrhizal symbiosis- Science 318: (#5848, 10/12) 265-268

Gadkar V., R. David-Schwartz, T. Kunik, Y. Kapulnik- 2001-Arbuscular mycorrhizal fungal colonization. Factors involved in host recognition- Plant Physiology 127: (#4) 1493-1499

Poulton J.L., R.T. Koide, A.G. Stephenson- 2001-Effects of mycorrhizal infection and soil phosphorus availability on *in vitro* and *in vivo* pollen performance in *Lycopersicon esculentum* (Solanaceae)- American Journal of Botany 88: (#10) 1786-1793

Winither J.L., W.E. Friedman- 2007-Arbuscular mycorrhizal symbionts in *Botrychium* (Ophioglossaceae)- American Journal of Botany 94: (#7) 1248-1255

Some plants are parasitic, and so do not need to make chlorophyll or do photosynthesis. Here is an example of a **parasitic orchid**, *Epipactis helleborine,* that grows in the woods in this area:

Light M.H.S., M. MacConaill- 1991-Patterns of appearance in *Epipactis helleborine* (L.) Crantz- pgs. 77-87, in, T.C.E. Wells and J.H. Willems editors, <u>Population ecology of terrestrial orchids</u>- SPB Academic Publishers, The Hague, Netherlands

Salmia A- 1989-Features of endomycorrhizal infection of chlorophyll-free and green forms of *Epipactis helleborine* (Orchidaceae)- Annales Botanici Fennici 26: (#1) 15-26

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Salmia A- 1986-Chlorophyll-free form of *Epipactis helleborine* (Orchidaceae) in SE Finland- Annales Botanici Fennici 23: (#1) 49-57

The development and physiology of **root nodules** and how **nitrogen fixation** is done in them is an area of immense interest and economic importance.

Barker D.G., P. Gallusci, V. Lullien, H. Kahn, M. Ghérardi, T. Huguet- 1988- Identification of two groups of leghemoglobin genes in alfalfa (*Medicago sativa*) and a study of their expression during root nodule development- Plant Molecular Biology 11: 761-772

Kaló P., C. Gleason, A. Edwards, J. Marsh, R.M. Mitra, S. Hirsch, J. Jakab, S. Sims, S.R. Long, J. Rogers, G.B. Kiss, J.A. Downce, G.E.D. Oldroyd- 2005- Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators- Science 308: (6/17) 1786-1789

Liang Y., J.M. Harris- 2005-Response of root branching to abscissic acid is correlated with nodule formation both in legumes and nonlegumes- American Journal of Botany 92: (#10) 1675-1683

Murray J.D., B.J. Karas, S. Sato, S. Tabata, L. Amyot, K. Szczyglowski- 2007-A cytokinin perception mutant colonized by *Rhizobium* in the absence of nodule organogenesis- Science 315: (1/5) 101-104

Oldroyd G.E.D- 2007-Nodules and hormones- Science 315: (1/5) 52-53

Streeter J.G- 1992-Analysis of apoplastic solutes in the cortex of soybean nodules- Physiologia Plantarum 84: 584-592

Tirichine L., N. Sandal, L.H. Madsen, S. Radutoiu, A.S. Albrektsen, S. Sato, E. Asamizu, S. Tabata, J. Stongaard- 2007-A gain-of-function mutation in a cytokinin receptor triggers spontaneous root nodule organogenesis- Science 315: (1/5) 104-107

Udvardi M.K., D.A. Day- 1997-Metabolite transport symbiotic membranes of legume nodules- Annual Review of Plant Physiology and Plant Molecular Biology 48: 493-523

Van de Velde W., G. Zehirov, A. Szatmari, M. Debreczeny, H. Ishihara, Z. Kevei, A. Farkas, K. Mikulass, A. Nagy, H. Tiricz, B. Satiat-Jeunemaître, B. Alunni, M. Bourge, K-i. Kucho, M. Abe, A. Kereszt, G. Maroti, T. Uchiumi, E. Kondorosi, P. Mergaert- 2010-Plant peptides govern terminal differentiation of bacteria in symbiosis- Science 327: (#5969, 2/26) 1122-1126

Wang D., J. Griffitts, C. Starker, E. Fedorova, E. Limpens, S. Ivanov, J. Bisseling, S. Long- 2010-A nodule-specific protein secretory pathway required for nitrogen-fixing symbiosis- Science 327: (#5969, 2/26) 1126-1129

The flip side of interacting with good microbes is dealing with those that are your pathogens. Here is an article that notes the presence of an **innate immune system** in plants based on their ability to detect bacteria that live in/on themselves. In this case rice detects an item secreted by a phytopathogenic bacterium.

Lee S-W., S-W. Han, M. Sririyanum, C-J. Park, Y-S. Seo, P.C. Ronald- 2009-A type 1- secreted sulfated peptide triggers Xa21-mediated innate immunity- Science 326: (#5954, 11/6) 850-853

And of, course, **carnivorous plants** are just neat:

Cameron K.M., K.J. Wurdack, R.W. Jobson- 2002-Molecular evidence for the common origin of snap-traps among carnivorous plants- American Journal of Botany 89: (#9) 1503-1509

Heslop-Harrison Y- 1978-Carnivorous plants- Scientific American 238: (#2) 104-115

Kato M- 1993-Floral biology of *Nepenthes gracilis* (Nepenthaceae) in Sumatra- American Journal of Botany 80: (#8) 924-927

Newell S.J., A.J. Nastase- 1998-Efficiency of insect capture by *Sarracenia purpurea* (Sarraceniaceae) the northern pitcher plant- American Journal of Botany 85: (#1) 85-91

Owen T.P.jr, K.A. Lennon- 1999-Structure and development of the pitchers from the carnivorous plant *Nepenthes alata* (Nepenthaceae)- American Journal of Botany 86: (#10) 1382-1390

Richards J.H- 2001-Bladder function in *Utricularia purpurea* (Lentibulariaceae): Is carnivory important?- American Journal of Botany 88: (#1) 170-176

BIO 107   2010
Day 8, Lecture 18, Title: Excretion
Text Readings: Campbell et al. (2008), chapter 44.

Topics to cover:

       Removing Salts
       Nitrogenous Wastes
       Some common features in most Animal excretion
       Planaria - Flame Cell System
       Insects - Malpighian Tubules
       Earthworms - Nephridia
       Mammals - Nephrons and Kidneys

We excrete for several reasons. (How does excretion differ from elimination?)
       One is to remove unwanted salts or wastes, while retaining desired items.
       Another is to carry out osmoregulation.
            To make the composition of osmotica in one compartment distinct from another.
                Think of: cytosol vs. intercellular fluid.
                                 blood serum vs. cytosol.
                                 body fluids vs. ocean.
We will review a few examples of organisms that carry out excretion.

Removing Salts
Salt glands in plants
              water is evaporated away, so salts remain. Including Nickle, Cadmium, arsenic..
              salts and ions left in the leaves, dropping leaves each year helps remove them?
              either accumulate in vacuoles, either in cells in leaf or in leaf hair cells
                     So if herbivores eat the leaf they get toxic salts!
                     There is a limit to how much can be accumulated this way.
              Excess often is secrete to leaf surface, rain then washes it off.
              Fig. 26.8 (Salisbury and Ross, 1992) saltgland1.jpg
              Fig. 26.9 (Salisbury and Ross, 1992) saltgland2.jpg

Salt glands in birds.
       In some species see a counter current systems,
            recall advantages compared to con-current system
       Bird salt glands as an example, get salt in sea water and marine fish they eat as food
            Fig. 44.7, Salt glands in birds
            Fig. 44.8, bird salt gland
            compare salt concentration in blood to in tubule
                 high in artery, low in vein, low at start of tubule, higher in central duct
            move salts by active transport as well.

note the need for tight junctions between the cells of tubules
        otherwise get leaks between cells   (an apoplastic barrier)
(will see later that plants have to do something similar, at casparian strip in roots)
With this structure movement of salts is across membranes of living cells,
        not between cells, so it can be controlled.

Nitrogenous Wastes

Amino acids and nucleotides are respired to CO_2. But what to do with the nitrogen?
So respiration of some organic matter produces nitrogenous wastes.
Review nitrogenous wastes, types, costs and benefits
Fig. 44.9, N waste types
        This figure suggests one species makes just one waste type, not really this way...
                rather, a species may just use one type much more than the others...
        ammonium, toxic at low concentrations, soluble, cheap
        urea, less toxic so can be at moderate concentrations, soluble, moderate cost
        uric acid, non-toxic, not soluble so forms crystals, expensive
Relate osmoregulation to soluble nitrogenous wastes.
        If accumulate urea, it acts as an osmoticum. excrete urea, water follows by osmosis.
        So to get rid of soluble wastes can get into water issues.
                Often need to recover the water.

Some common features in most Animal excretion

Nephridia as a common structure, though not in all
Some functions are often seen...
Fig. 44.10, key functions of nephridia, filter, abs, sec.
                filtration,   reabsorption, secretion,   excretion
        filtration is often a physical process. Pressure driven, and may filter by size.
                The pressure gradient is across the tight junctions
                        NOT across any plasma membranes as the text (fig. 44.10) implies
                Why use filtration, why not use just secretion?
                Consider: Can your cells recognize all the toxins you eat?
        reabsorption of items from the filtrate. Via membrane transport systems.
        secretion via transport systems into the filtrate.
        excretion is the final removal of the modified filtrate (urine) out of the body.
Need to balance osmotica (solutes and water), and deal with metabolic wastes

Planaria - Flame Cell System
        Fig. 44.11, Planaria, protonephridia
            (Again, the fluids here do not cross a membrane, as this figure suggests?)
        no circulatory system, flat and thin
        flame cells secrete, and cells lining allow passage
            of wastes into tubules, intercellular fluid can leak in, filtration?
            The fluids move between cells due to leaky connections.
            The flagella of flame cells push the fluid along.
        Wastes then leave by body pore
        live in freshwater, must retain salts, not worried about water retention
        Given lots of water, which N-waste would they use?

Insects - Malpighian Tubules
      Insects, Malpighian tubules
      Fig. 44.12, Malpighian tubules of insects
        open circulatory system, low blood pressure, so no filtration
        active secretion into the tubules, water follows by osmosis
            this creates osmotic pressure in a dead-end tubule
            produces pressure in tubules (will see this is like phloem in plants)
        Malpighian tubules then open out into digestive tract, so pressure flows to there
        recovery of salts and water in rectum, potential for counter current here?
        feces very dry, uric acid, very good water recovery done here
        Why would insects need such a very efficient water recovery system that they
            would pay the costs of using uric acid?

Earthworms - Nephridia
      Fig. 44.12, earthworm, metanephridia
        closed circulation
        capillary bed about nephron to reabsorb items
        opening to nephridium from internal body space
            pressure from hydrostatic skeleton pushes fluid in
        so non-specific body fluids enter (some filtration present)
        reabsorption by cells lining collecting tubule
            then the items are put back into blood via capillaries.
        urine goes into bladder, then out
        lives in moist soil, so not worried so much about water retention
        So which N-waste is it likely to use?

Mammals - Nephrons and Kidneys
      Humans/mammalian system has many nephridia in the kidney
      Fig. 44.14, human excretory system
        Note blood flow, aorta, to renal artery, so a high blood pressure
        go over parts of nephron
      Fig. 44.15, nephron
        note sites of:

filtration, secretion, reabsorption

note water permeability low in ascending limb of loop of Henle

Fig. 48.8, (Purves etal., 1998) nephron_SEM.jpg

note Bowman's capsule, this is a capillary bed

note glomerulus, and filtration that is done

recall we covered loss of fluid from a capillary bed, here it is a benefit

Fig. 44.15, human nephron functioning

Note osmolarity gradients (what is osmolarity?) in kidney

active secretion of items, reabsorption of items to blood

blood leaves at about 300 mosM, urine leaves at about 1200 mosM

So a concentrated urine is produced.... why? Consider water recovery.

What form of N-waste do we mainly use?

Note that blood flow as leaves is countercurrent to urine flow

Fig. 44.14, Human excretory system

consider why the veins must pass back through the cortex of the kidney

What would happen if the exited from the medulla of the kidney?

There is another counter current system based on flow in the nephron

Consider the loop of Henle vs. the flow in the collecting duct.

This helps to maintain the osmotic gradient in the kidney.

Different species can alter nephron and alter water recovery

Can lengthen or shorten the loop of Henle.

Can alter the osmotic gradient the nephron experiences.

Counter current systems are good at promoting exchange, and at

maintaining a gradient. In the kidney we see both of these.

note there is a portal system, glomerulus to vasa recta...

these are two capillary beds connected by a portal vein.

Where in mammals have we seen a portal system before?

What does this do to the rate of blood flow? Is that good or bad?

What parts/functions of the nephron are regulatable?

Could the filtration be altered? If so, how?

How would you alter secretion?

How would you alter reabsorption of an item?

Can the rate of water recovery be changed?

Hormonal regulation of the kidney
>Fig. 44.19, ADH control of the nephron
>ADH = antidiuretic hormone
>Cells in brain detect rise in blood osmolarity,
>>this triggers hypothalamus to signal for ADH release from the pituitary
>Reception of ADH, by cells in the collecting duct of the nephron
>promotes vesicles with aquaporins to fuse with plasma membrane
>Aquaporins are water channels, this increases water permeability
>>so the osmotic gradient can pull water back out of urine better
>>more water is recovered, less stays in urine
>So this is a homeostatic system for control of blood osmolarity...

In various degrees these excretory systems act to:
>remove toxins (N-wastes, etc)
>recover/rebalance organic molecules and ions
>recover water

Objectives:

Compare a counter-current system to a con-current system in terms of their efficiency for the removal or recovery of an item. Be able to identify examples from various species that illustrate this comparison.

In terms of the environment in which they live and the activities they carry out describe why both plants and sea birds must deal with excess salts. Be able to contrast the means by which they do so.

The functions of filtration, secretion and reabsorption are carried out in mammalian nephridia. Be able to describe each process, and how it contributes to the formation of a concentrated urine. What aspects of these functions are seen in the excretory systems of planaria, earthworms, or insects? Compare the benefits and costs of using either urea, ammonia or uric acid as a nitrogenous waste in terms of their costs to produce, their solubility, and their toxicity. Be able to compare planaria, earthworms, insects and mammalian excretory systems. Which of these animals has to recover water most efficiently, and how is this need met in terms of the excretory system they have and the type of waste they excrete? When an organism carries out excretion what is it trying to achieve? How does this differ from the process of elimination?

Know the anatomy of the mammalian kidney and nephron so that you can describe the place and function of each part in the processes it carries out (structures mentioned in figure 44.14 are relevant). Review the path of blood flow from the heart to the kidney and back. How does the blood pressure change as blood passes from the renal artery past the nephron to the renal vein? How many capillary beds does this blood pass through and where are they located? How are blood and filtrate flows orientated in the mammalian kidney to maintain the osmotic gradient between the cortex and medulla of the kidney? Be able to describe how ADH acts in the nephron to alter water recovery.

For review see self-quiz questions #1-6 for chapter 44.

Needed overheads and items:

Fig. 26.8 (Salisbury and Ross, 1992) saltgland1.jpg
Fig. 26.9 (Salisbury and Ross, 1992) saltgland2.jpg

Fig. 44.7, Salt glands in birds
Fig. 44.8, Bird salt gland
Fig. 44.9, N waste types
Fig. 44.10, key functions of nephridia, filter, abs, sec.
Fig. 44.11, Planaria, protonephridia
Fig. 44.13, Malpighian tubules of insects
Fig. 44.12, earthworm, metanephridia
Fig. 44.14, human excretory system
Fig. 44.15, nephron
Fig. 48.8, (Purves etal., 1998) nephron_SEM.psf
Fig. 44.16, human nephron functioning
Fig. 44.14, human excretory system
Fig. 44.19, ADH regulation of the nephron

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Osmoregulation and excretion. Chapter 44. Pages 954-974. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Purves W.K., G. H. Orians, H.C. Heller, D. Sadava- 1998-Life: The Science of Biology. 5th edition. Figure. 48.8. Sinauer Associates Inc. Salt Lake City, Utah

Salisbury F.B., C.W. Ross- 1992- Plant Physiology, 4th edition. Figures 26.8, 26.9. Wadsworth Publishing Co., Belmont, CA.

Related issues:

Many species other than animals also respond to osmotic stress and must carry out osmoregulation.

Osmoregulation in **plants**:

Allen G.J., D. Sanders- 1994-Osmotic stress enhances the competence of *Beta vulgaris* vacuoles to respond to inositol 1.4.5-triphosphate- The Plant Journal 6: (#5) 687-695

Heuer B- 1989-Photosynthesis and osmotic adjustments of two sugar beet cultivars grown under saline conditions- Journal of Experimental Botany 40: (#213) 437-440

Homann U- 1998-Fusion and fission of plasma-membrane material accomodates for osmotically induced changes in the surface area of guard-cell protoplasts- Planta 206: 329-333

Keller C.P., E. VanVolkenburgh- 1996-Osmoregulation by oat coleoptile protoplasts: Effect of auxin- Plant Physiology 110: 1007-1016

Meissner S.T., R.M. Spanswick- 1994-Growth of storage organ and parenchyma cells in red beet (*Beta vulgaris* L.): Lower osmolarity correlates with increasing cell size, implying cell transport rather than diffusion limitation- International Journal of Plant Science 155: (#1) 36-48

Patrick J.W- 1993-Osmotic regulation of assimilate unloading from seed coats of *Vicia faba*. Assimilate partitioning to and within attached seed coats- Physiologia Plantarum. 87: 345-352

Talbott L.D., E. Zeiger- 1996-Central roles for potassium and sucrose in guard-cell osmoregulation- Plant Physiology 111: 1051-1057

Osmoregulation in **algae**:

Kiyosawa K., K. Ogata- 1987-Influence of external osmotic pressure on water permeability and electrical conductance of *Chara* cell membrane- Plant Cell Physiology 28: (#6) 1013-1022

Oren-Shamir M., U. Pick, M. Avron- 1990-Plasma membrane potential of the alga *Dunaliella*, and its relation to osmoregulation- Plant Physiology 93: 403-408

Osmoregulation in **yeast** has been studied, including the signal transduction done during it:

Mettetal J.T., D. Muzzey, C. Gómez-Uribe, A. van Oudenaarden- 2008-The frequency dependence of osmo-adaptation in *Saccharomyces cerevisiae*- Science 319: (#5862, 1/25) 482-484

Osmoregulation also is done in **prokaryotes**:

White D- 2000-Homeostasis- Chapter 15, pgs. 384-397, in, The Physiology and Biochemistry of Prokaryotes. 2nd edition. Oxford University Press, N.Y.

For more on **osmoregulation** in **animals** see:

Eckert R., D. Randall- 1978-Osmoregulation and excretion- Chapter 12, pgs. 392-432, in Animal Physiology. W.H. Freeman and Company, San Francisco

Hildebrand M., G. Goslow- 2001-Excretory system and osmoregulation. Chapter 15, pgs 271-281, in Analysis of Vertebrate Structure. 5th edition. John Wiley & Sons, Inc. N.Y., N.Y.

Wigglesworth V.B- 1984-Excretion- Chapter 5, pgs. 69-88, in Insect Physiology. Chapman and Hall Publishers, London.

Many **fish** make calcium carbonate as a means to free up water as a part of their osmoregulation. This affects the carbon cycle in the ocean.

Seibel B.A., H.M. Dierssen- 2009-Animal function at the heart (and gut) of oceanography- Science 323: (#5912, 1/16) 343-344

Wilson R.W., F.J. Millero, J.R. Taylor, P.J. Walsh, V. Christensen, S. Jennings, M. Grosell- 2009-Contribution of fish to the marine inorganic carbon cycle- Science 323: (#5912, 1/16) 359-362

Some **pathogens** act by inducing breakdown in osmoregulation in their host cells.

Hutchison M.L., M.A. Tester, D.C. Gross- 1995-Role of biosurfactants and ion channel-forming activities of syringomycin in transmembrane ion flux: A model for the mechanism of action in the plant-pathogen interaction- Molecular Plant-Microbe Interactions 8: (#4) 610-620

BIO 107   2010
Day 8, Lecture 19, Title: Movement I: Muscles.
Text Readings: Campbell et al. (2008) pgs. 112-118, 558-559, 838, 842-843, 1105-1114.

Topics to cover:

      Generation of Force
      Flagellar systems
      Plant system
      Hydrostatic skeletons
      Vertebrate muscles
            Sarcomere functioning
            Regulation of Muscular Action
      Summary

Generation of Force
To move we need force generation, anchorage to push/pull against, and regulation
      (this is a stimulus/response system...)
Three broad systems:
      One based on use of cytoskeletal elements (microtubules or microfilaments, etc..)
      One uses proton motive force to power twisting of extracellular matrix
      And another is based on osmotic pressure generation
Here we will talk mainly about cellular systems
      in later lectures we will cover:
            muscles and bones in vertebrates
            how neurons stimulate muscle cells
            plant use of pressure for long distance internal transport

Flagellar systems
Role of the cytoskeleton
      Fig. 6.27, motility
            common feature of muscle sarcomere, pseudopods, cyclosis all cytoskeletal
      microtubules and microfilaments make a structure for anchorage
      motor proteins move over this structure to generate force
            for microtubules the motor protein is often dynein (or others)
            for microfilaments the motor protein is typically actin
Eukaryotic cilia and flagella
      Fig. 6.24, ultrastructure of eukaryotic cilia and flagella
      Fig. 6.25, how dynein walks up microtubuoles
      Note structure and parts: membrane covered, tubulin and dynein
      motor protein (dynein) is an ATPase, ATP hydrolysis coupled to change in protein shape
            able to bind to microtubules and so shape change becomes force generation

Bacterial flagella
 Fig. 27.6, bacterial flagellum
 recall ATP synthase in mitochondria and chloroplast, it rotates
 Fig. 14.17, (Alberts et al. 2002) flagellum_bacterial.jpg
  note pump makes pH gradient
  flagellum is like reverse pump, uses energy in proton motive force to rotate
  flagellin is extruded protein, is in extracellular matrix
 Note how different from eukaryotic flagellum...
  Still has force generation, by twisting
  still has structure to push against, held by membranes and bacterial cell wall.
  anchorage is in extracellular matrix, motor protein here is not an ATPase

Plant system
 Fig. 39.26, *Mimosa pudica* pulvinus closing and opening
 Not based on cytoskeleton, rather it is Osmosis based
 whole cells swell and shrink, generate force
 How do you make a cell swell?
  Fig. 36.9, turgid cells in plants
  Draw cell, with more K^+ in than out, $\Delta Em = -70$ mV
  To take up K^+, what must happen to membrane potential?
  H^+-ATPase pumps H^+ out to generate membrane potential
  Is this hyperpolarizing or depolarizing the membrane?
  Moving ions, drives osmosis, generates pressure, changes cell size...
 This is under control of plants, so it is regulated
  accounts for "sleep movements" of leaves
  Fig. 39.20, bean plant, sleep movements
 How then would you make this cell shrink?
 Role of membrane transport systems helps make force at cellular, tissue, organ levels.
 So plants change the pressure inside a fluid-filled space.
 See something similar in some animals...

Hydrostatic skeletons
 Hydrostatic skeletons in animals are fluid filled spaces with changes in pressure
 Consider the earthworm
  Fig. 50.33, earthworm, movement with hydrostatic skeleton
  note longitudinal and circular muscles
  pressure produced here is not due to osmosis, but due to the muscles
  pull on outer body wall, which pushes on fluid
  fluid pushes back on body wall in all directions
 a benefit of tissues, tight cell junctions, so something seen with multicellularity...
 Examples of hydrostatic skeletons in humans are...?

Vertebrate muscles
Endoskeletal muscle attachments vs. Exoskeletal
        Insects have exoskeleton, we have an endoskeleton.
        Fig. 50.32, endo vs. exoskeletal muscle attachments
        skeleton for anchorage, muscles for force generation
        compare extensors and flexors in each case
                we will have more on this later in the Movement II lecture....
                today we will focus mainly on the action at the muscle cell level.
Vertebrate muscle types, review the basic types and where located
        Fig. 40.5, muscle types
        smooth, slow contraction, multidirectional, lines vessels, intestinal tract, involuntary
                So a muscle need not be fast or unidirectional
        cardiac, are more directional, intermediate in speed, but sometimes self timed, involuntary
                quicktime movie, 16.4, (Alberts etal, 2002a; CD)
                so two cells can coordinate with each other!
        skeletal, unidirectional fast contraction, skeletal muscle cells are typically voluntary
Skeletal muscles are our focus
        Fig. 50.25, muscle to sarcomere, level of organization
        note need for attachments: tendons attach muscles to anchoring bones
                so need cell-to-cell and cell-to-extracellular matrix connections
        fiber = single cell, myofibril, sarcomere, multinucleate
                one cell through one end of bundle to the other
        Note have structure to pull on: microfilaments (thin filaments)
                have motor proteins to generate force: myosin (thick filaments)
        Fig. 16.70, myofibril cross section (Alberts etal, 2002b) myofibril_cs.jpg
                note appearance in cross section, previous is longitudinal section
                      things look different in various sectioning

Sarcomere functioning
Sarcomeres are the basic repeating unit in the myofibrils. In serries along length.
Note the Z line. It is a protein structure and acts as anchor for microfilaments (actin)
The thick filaments are groups of muscle myosin (the motor proteins)
Fig. 50.26, sliding filament, sarcomere
        TEMs of sarcomere
        note how appearance changes with contraction
        the motor proteins pull the structure inwards

Actin/myosin interaction

      Fig. 50.27, myosin/actin interaction

            microfilaments are made up actin, passive anchorage structure

            thick filaments are made up of muscle myosin, the motor protein

            note coupled reactions: ATP cleavage, and change in protein shape

            Note: references to myosin in "high" and "low" energy states in this figure are a bit misleading. Proteins do not store energy, but tend to spontaneously move to lowest energy state.

      quicktime movie, 16.6, (Alberts etal, 2002a; CD)

            actin/myosin animation, Shows change in shape well, a model

      quicktime movie, 16.8, (Alberts etal, 2002a; CD)

            crawling actin expt.

            isolated myosin and attach to glass, then give microfilaments and ATP

            able to put label on actin, so fluoresces, can then study interaction *in vitro*

Regulation of Muscular Action

A site of control of contraction, we will see steps in signal transduction that can be altered.

      Fig. 50.28, control of muscle contraction

            tropomyosin covers sites on actin where myosin would bind.

            troponin, when bound to Ca^{+2} shifts tropomyosin off, so changes in shape here

      Where does Ca^{+2} come from?

            Fig. 50.29, neuromuscle junction

                  note location of neuron, a motor neuron

                  neuron signals to muscle cell (will cover synapse actions in later lecture...)

                  Ca^{+2} stored in sarcoplasmic reticulum, the blue sheets

                  note location of: sarcomere, troponin and tropomyosin, myofibrils

      Organization at cell/tissue level

            Fig. 50.30, skeletal muscle and junction

                  note one muscle cell (i.e. a muscle fiber), arranged in a muscle tissue...

                  one neuron can attach to several muscle cells

                  so muscle cells can act in units... motor units

      Now back to the subcellular level, how do we get from

            neuron signal to muscle cell contraction?

            Fig. 50.29, muscle contraction

                  Acetylcholine receptors bind ACh that the motor neuron released

                  This causes sodium ion channels to be opened

                  Ion channels let Na^{+} cross the membrane, the membrane depolarizes

                  Membrane potential shift is across membrane, including down T tubules

                  Shifts in electrical field causes proteins in T tubules to change shape

             These proteins interact with proteins in the nearby sarcoplasmic reticulum

                  This changes activity of Calcium ion channels in SR

                  Calcium ions move into the cytosol, and diffuse to the myofibrils

In the myofibrils the calcium ion concentration rises in sarcomere
    Troponin binds calcium ions, it changes shape and shifts tropomyosin,
    microfilament's site for myosin interaction is exposed
    actin/myosin cycle proceeds
Notice what is happening in the T tubules as they interact with the SR
Fig. 16.73, T tubules and Ca^{+2} release (Alberts etal, 2002b) sarcoplasmic_reticulum.jpg
    The membrane potential change is an electrical field, so travels at the speed of light
    Notice how the voltage-sensing protein is directly influencing the ion channel
        This is a fast interaction, no secondary messengers...
To recover: Take Ca^{+2} back into SR, need ion pumps, close the ion channels,
        have the membrane potential recover, tropomyosin covers sites again
    So to recover have to move Ca^{+2} back into SR. How would this be done?
        Also need to make ATP, that involves respiration.

Alterations to the system
    Fig. 50.25
    To strengthen pull: more myofibrils in parallel in the muscle cell.
    To quicken pull: alter rate of signal from membrane to sarcomere.
    What happens when you die?
        No oxygen gas? ATP?
        Can myosin interact with actin?
        At what point in the cycle does it halt?

Summary
    Anchorage, structure to work on
        extracellular matrix at cellular or above...
        cytoskeleton
    Force generation
        by osmosis
        by motor proteins on cytoskeleton
        by pmf driven twisting of ECM
    Different ways to achieve this in various forms of life
    Allows movement of parts of body or entire body...

Objectives:

For a given system that results in movement be able to identify the structure acting as anchorage, and be able to identify the molecular systems by which force is generated.

Be able to describe the role of cytoskeletal structures in movement. What does a motor protein do, and what are examples of motor proteins?

Be able to contrast the means by which single-celled organisms achieve movement versus ways in which multi-celled organisms manage to move. What are three ways in which the eukaryotic flagellum differs from a bacterial flagellum? In eukaryotic flagella which motor protein moves along which static cytoskeletal element? What are the analogous molecular items seen in a sarcomere of a skeletal muscle cell?

If an earthworm wishes to extend forward what muscles must it contract? If an earthworm wishes to pull back what muscles must it contract? What is a hydrostatic skeleton?

How do plants manage to move parts of their bodies? How do they generate the forces needed to achieve this movement?

Know the characteristics of each of the three major vertebrate muscle cell types.

Be able to describe the basic structures of a skeletal muscle from the organ level down through the cellular level and on to the arrangement of the major molecules in the sarcomere. What is the sequence of events that occur as muscle myosin interacts with actin in a sarcomere during muscle contraction? Know how this interaction is regulated, including how a signal received at the cell membrane results in the contraction of a skeletal muscle cell (fig. 50.29 might be helpful here). Be sure to note the role that calcium ions play in this process and the proteins with which they interact to influence the cell's response. What could be altered to make the tension generated by a muscle greater, or make it operate faster?

For review see self-quiz question #6 of chapter 50.

Needed overheads and items:

Fig. 6.27, motility
Fig. 6.24, ultrastructure of eukaryotic cilia and flagella
Fig. 6.25, how dynein walks up microtubuoles
Fig. 27.6, bacterial flagellum
Fig. 14.17, (Alberts et al. 2002) flagellum_bacterial.jpg
Fig. 39.26, *Mimosa pudica* pulvinus
Fig. 36.9, turgid cells in plants
Fig. 39.20, bean plant, sleep movements
Fig. 50.33, earthworm, movement with hydrostatic skeleton
Fig. 50.32, endo vs. exoskeletal muscle attachments
Fig. 40.5, Muscle tissue types
quicktime movie, 16.4, (Alberts etal, 2002a; CD)
Fig. 50.25, muscle to sarcomere, level of organization
Fig. 16.70, myofibril cross section (Alberts etal, 2002b) myofibril_cs.jpg
Fig. 50.26, sliding filament, sarcomere
Fig. 50.27, myosin/actin interaction
quicktime movie, 16.6, (Alberts etal, 2002a; CD)
quicktime movie, 16.8, (Alberts etal, 2002a; CD)
Fig. 50.28, control of muscle contraction
Fig. 50.29, neuromuscle junction
Fig. 50.30, skeletal muscle and junction
Fig. 50.29, muscle contraction
Fig. 16.73, T tubules and Ca^{+2} release (Alberts etal, 2002b) sarcoplasmic_reticulum.jpg
Fig. 50.25, muscle to sarcomere, level of organization

As Handout:
Fig. 14.17, (Alberts et al. 2002) flagellum_bacterial.jpg
Fig. 16.73, T tubules and Ca^{+2} release (Alberts etal, 2002b) sarcoplasmic_reticulum.jpg

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002a-<u>Molecular Biology of the Cell: Cell Biology Interactive CD</u>. Figures 16.4, 16.6, 16.8. Garland Science Publishing. N.Y., N.Y.

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002-<u>Molecular Biology of the Cell</u>. 4th edition. Movies 14.17, 16.70, 16.73. Garland Science Publishing. N.Y., N.Y.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Sensory and Motor Mechanisms. Chapter 50. Pages 112-118, 558-559, 838, 842-843, 1105-1114. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Related issues:

For more on **muscles** see:

Hildebrand M., G. Goslow- 2001-Muscles and Electric Organs. Chapter 10, pgs 169-193, in <u>Analysis of Vertebrate Structure</u>. 5th edition. John Wiley & Sons, Inc. N.Y., N.Y.

Wigglesworth V.B- 1984-Muscles and movement- Chapter 9, pgs. 145-153, in <u>Insect Physiology</u>. Chapman and Hall Publishers, London.

With **aging** our muscles change. Here is a study that notes several changes in the cells that make up our muscles as we grow older.

Brack A.S., M.J. Conboy, S. Roy, M. Lee, C.J. Kuo, C. Keller, T.A. Rando- 2007-Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis- Science 317: (#5839, 8/10) 807-810

The structure of the **sarcomere** is based on use of the cytosketon. For more on its structure and the cytoskeletal features, such as myosin, and how they are studied see:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walker- 2002-The cytoskeleton. Chapter 16, pgs. 907-982, in <u>Molecular Biology of the Cell</u>. 4th edition. Garland Science, N.Y., N.Y.

Kron S.J., J.A. Spudich- 1986-Fluorescent actin filaments move on myosin fixed to glass surface- Proceedings of the National Academy of Science (USA) 83: 6272-6276

Shiroguchi K., K. Kinosita jr.- 2007-Myosin V walks by lever action and Brownian motion- Science 316: (5/25) 1208-1212

In **electric fish** it is the membrane potential of the muscles that produce the electric shock. Here is a source that describes how this field is produced by muscle tissue:

Shepherd G.M- 1988-Molecular neurobiology- Chapter 2, pgs. 13-38, in <u>Neurobiology</u>, 2nd edition. Oxford University Press, N.Y.

The muscle cell has to alter its membrane potential to initiate and control contraction. This is done by moving ions across its the plasma membrane. This ion movement has been studied through the individual **ion channels** some that are specific for sodium ions, others for other ions.

Sigworth F.J., E. Neher- 1980-Single Na$^+$ channel currents observed in cultured rat muscle cells- Nature 287: 447-449

White R.C., T.S. Elton, R.L. Shoemaker, T.A. Brock- 1995-Calcium-sensitive chloride channels in vascular smooth muscle cells- Proceedings of the Society for Experimental Biology and Medicine 208: 255-262

For some examples of movement in higher life forms that are based on **hydrostatic** forces see the the following:

Applewhite P.B., F.T. Gardner- 1971-Rapid leaf closure in *Mimosa* in response to light- Nature 233: 279-280

Darwin C- 1881-The Power of Movement in Plants- D. Appleton and Co. N.Y., N.Y. 592 pgs. (1996 reprint, by DeCapo Press.)

If you wish to know how a muscle works you might study it by growing it in isolation. So studies of how to **grow muscular organs**, start with how to grow individual cells might tell us something about muscles in us as well.

van Rooij, E.L.B. Sutherland, X. Qi, J.A. Richardson, J. Hill, E.N. Olson- 2007-Control of stress-dependent cardiac growth and gene expression by a microRNA- Science 316: (4/27) 575-579

BIO 107   2010
Day 8, Lecture 20, Title: Plant Dispersal
Text Readings: Campbell et al. (2008), pgs. 804-805, 809-811.

Topics to cover:

Show Private life of plants, volume I

Pre video:
Plants are sessile, their individuals do not move much.
       Still have to disperse their species.
       (Same problem animals solve by individual movement?)
       Various plant species disperse spores or seeds.

Note: Pollination is NOT dispersion.
       Pollination MUST get to where another member of the same species is located.
       Disperal is all about getting to new places the species had never been.

Keep track of mechanisms of dispersal that plants use

       Abiotic means
              such as, wind, water

       Biotic means
              By hiring animals
                     On surface of animal, external
                     Through the animal's interior?l

Watch video.... (popcorn optional....?)

Objectives:

Be able to describe several mechanisms by which plants manage to disperse their offspring even though established plants themselves tend not to migrate. Which mechanisms are biotic and which involve abiotic means? Consider the effect of each on the range in which a plant species would be found. What can a plant do to promote biotic means of dispersal? What modifications might be expected to help promote abiotic dispersal?

Some mechanisms of dispersal are rather harsh and relatively risky for the plant's seeds. Describe the various benefits a plant species obtains from running such risks to achieve good dispersal.

Be sure to understand what pollination is, and how it is not a means of dispersing a species.

Needed overheads and items:

The Private life of plants: Volume I: Branching out
    David Attenborough

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Angiosperm reproduction and biotechnology. Chapter 38. Pages 804-805, 809-811. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Attenborough D- 1995-<u>The Private Life of Plants: A natural history of plant behaviour</u>. Pgs. 11-40. Princeton University Press. Princeton, N.J.

Attenborough D- 1995-*The Private Life of Plants*, Volume I: *Branching out.* British Broadcasting Corporation.

Related issues:

Some articles relating to **abiotic** means of plant dispersal:

Andersen M.C- 1993-Diaspore morphology and seed dispersal in several wind-dispersed asteraceae- American Jouranl of Botany 80: (#5) 487-492

Elbaum R., L. Zaltzman, I. Burgert, P. Fratzl- 2007-The role of wheat awns in the seed dispersal unit- Science 316: (5/11) 884-886

Garrison W.J., G.L. Miller, R. Raspet- 2000-Ballistic seed projection in two herbaceous species- American Journal of Botany. 87: (#9) 1257-1264

Lentink D., W.B. Dickson, J.L. van Leeuwen, M.H. Dickinson- 2009-Leading-edge vortices elevate lift autorotating plant seeds- Science 324: (#5933, 6/12) 1438-1440

Matlack G.R- 1987-Diaspore size, shape, and fall behavior in wind-dispersed plant species- American Journal of Botany 74: (#8) 1150-1160

Miller N.G., S.F. McDaniel- 2004-Bryophyte dispersal inferred from colonization of an introduced substratum on Whiteface Mountain, New York- American Journal of Botany 91: (#8) 1173-1182

Sipe T.W., A.R. Linnerooth- 1995-Intraspecific variation in samara morphology and flight behavior in *Acer saccharium* (Aceraceae)- American Journal of Botany 82: (#11) 1412-1419

And some articles that consider **biotic** means of dispersal:

Griffin S.R., S.C.H. Barrett- 2004-Post-glacial history of *Trillium grandiflorum* (Melanthiaceae) in eastern North America: Inferences from phylogeography- American Journal of Botany 91: (#3) 465-473

Hooker J.J., M.E. Collinson- 2003-Plant-animal interactions: Dispersal- Chapter 4.1.18, pgs. 429-431, in Palaeobiology II. D.E.G. Briggs and P.R. Crowther editors. Blackwell Pub. Oxford, U.K.

Quinn J.A., D.P. Mowrey, S.M. Emanuele, R.D.B. Whalley- 1994-The 'foliage is the fruit' hypothesis: *Buchloe dactyloides* (Poaceae) and the short grass prairie of North America- American Journal of Botany 81: (#12) 1545-1554

Shea K- 2007-How the wood moves- Science 315: (3/2) 1231-1232

These articles study whether **dispersion limits** the spread of plant species into a new habitat:

Alsos I.G., P.B. Eidesen, D. Ehrich, I. Skrede, K. Westergaard, G.H. Jacobsen, J.Y. Landvik, P. Taberlet, C. Brachmann- 2007-Frequent long-distance plant colonization in the changing arctic- Science 316: (6/15) 1606-1609

Cain M.L., B.G. Milligan, A.E. Strand- 2000-Long-distance seed dispersal in plant populations- American Journal of Botany 87: (#9) 1217-1227

Primack R.B., S.L. Miao- 1992-Dispersal can limit local plant distribution- Conservation Biology 6: (#4) 513-519

Winn A.A- 1985-Effects of seed size and microsite on seedling emergence of *Prunella vulgaris* in four habitats- Journal of Ecology 73: 831-840

BIO 107   2010
Day 9, Lecture 21, Title: Hormones
Text Readings: Campbell et al. (2008), pgs. 207-210 and chap. 45.

Topics to cover:

       Definition of a Hormone
       Types of Hormones
       Homeostasis and Negative Feedback
           Single hormone example (Thyroid)
           Double hormone examples (Thyroid/Parathyroid and Pancreas)
       Hypothalamus and Pituitary
       Common Features of mammalian systems

Definition of a Hormone
       Fig. 11.5, common inter-cellular signaling pathways
       Operates within the body of the organism (contrast with pheromones?)
       acts at a distance from the place it is made.
           Secreted out of cells (contrast with secondary messengers?)
       In many animals another common feature is traveling through the blood stream
           which makes the hormone both a long distance and fast means of communication
           (contrast with synaptic and paracrine signaling?)
       There are hormones in all multicelled species. Obviously some lack a circulatory system,
           so movement through circulatory system is very much an animal-specific one.
Mammalian hormonal system
       Our main focus is the mammalian model. But principles apply to other groups as well
       Hormones travel through the blood, but arrangements can vary.
           Fig. 45.11, endocrine pathway
           Fig. 45.16, neurohormone pathway
           Fig. 45.18, neuroendocrine pathway
           Note that all these use the circulatory system, but differ
               use nervous system or not,
               have intermediate endocrine glands or not
       What releases the hormone out into the body? (contrast endocrine and exocrine?)
           Fig. 45.10, human endocrine glands
           hormones are secreted by endocrine glands into body fluids, i.e. blood serum
           note that this is not a full list of endocrine glands,
               some are both endocrine and exocrine, see pancreas...
       Table 45.1, list of endocrine glands and their hormones
           Do not memorize all of this, use it to organize around three types
               and as a source for the examples we do cover
           Three broad classes of mammalian hormones (other minor ones exist...)
               Derivatives from peptides or amino acids, such as amines.
               Proteins or glycoproteins.
               Steroids

Hormones and signal/response systems
   A hormone can be viewed as an intraorganismal signal.
   Cells that respond to the hormone have:
      receptor, transductive and response systems.
      Fig. 45.5, chemical signaling review
      This can involve the rapid, modification of activity of existing proteins
            Could modify uptake by the cell of some item in the blood serum.
         Or can lead to the slower making of make new proteins,
            So a slow response could be a change in growth...
      Note location of receptors, which is a hydrophobic hormone, which hydrophilic?
   Even same hormone, and same receptor can lead to different cellular responses
      Fig. 45.8, diverse responses, same signals
         epinephrine; flight or fight response
            vessel constriction, dilation, glycogen mobilization
         so three different responses by distinct cells to the same hormone

Types of Hormones
Consider examples of the three main classes of mammalian hormones.
Derived from amino acids
   Will cover two examples (do not worry about the structures of them...)
      Fig. 45.10, human endocrine glands
         epinephrine from adrenal medulla
         T_3 and T_4 from thyroid
   Epinephrine
      Fig. 45.12, (Campbell and Reece, 2002) catecholamine hormones
      epinephrine derived from the amino acid tyrosine
   Thyroid hormones T_3 and T_4
      Fig. 45.7, (Campbell and Reece, 2002) T3 and T4 hormones
      triiodothyronine (T_3), thyroxine (T_4) hormones
      they alter the metabolic rate (what does this mean..."metabolic rate"?)
      These have R groups altered while in a protein
         then protein is degraded and these are released
      Fig. 29.13, (Zubay, 1983) thyroxine.jpg

Whole protein hormones
   This implies that there is a gene coding for mRNA coding for a protein...
   Consider insulin, made by cells in the pancreas
      Fig. 45.10, human endocrine glands
      Fig. 29.10, (Zubay, 1983) insulin.jpg
      Note how modified by cleavage
         signal peptide is present for formation of it in the lumen of the ER
         disulfide bonds
         cut before secreted by Beta cells of the pancreas
Steroid hormones
   From the gonads, and the adrenal cortex

Fig. 45.10, human endocrine glands
Fig. 45.13, (Campbell and Reece, 2002) steroid hormones
steroids, glucocorticoid, mineralcorticoid
some of these are the sex hormones, hydrophobic, slower acting?
Table 45.1, list of endocrine glands and their hormones
note steroid hormones made by Adrenal cortex, gonads, slow responses
So hormones are diverse types of molecules
Fig. 45.3, hormone types
More or less water soluble.
Modified from amino acids, polypeptides, or steroids, and others...
Were chemicals like these used in single celled organisms before multicelled organisms?
Therefore, would the original ancestral eukaryotic species, from which all eukaryotes are
descended, have used any of these sorts of molecules?
Consider: given evolution, hormones found in fungi, plants should be like what....?
(Some plants contain phyto-estrogens... Which are like what...?)

Homeostasis and Negative Feedback
Negative feedback can be used to limit a response
Fig. 1.13, negative and positive feedback
Here the presence of an item promotes production of another item that acts to inhibit first
item's production. A self-limiting situation.
Unlike positive feedback... which produces some final change
this can be good for growth or development where a distinct change is desired
Recall an example of negative feedbace we saw in respiration
Fig. 9.21, control of cellular respiration
need to keep systems from swinging to extremes: achieve homeostasis
Two broad ways to use hormones to achieve homeostasis
one at a time, or in antagonistic pairs... will consider both as examples

Single hormone example (Thyroid)
Thyroid
Fig. 45.10, human endocrine glands
Fig. 45.9, negative feedback, thyroid hormones (Campbell and Reece, 2005)
Fig. 45.18, neuroendocrine pathway, thyroid hormones
regulation of metabolic rate via T_3 and T_4
Note T_3 and T_4, also to control the concentration of these need:
Thyroid releasing hormone (TRH)
thyroid stimulating hormone (TSH)
and hypothalamus and anterior pituitary of the brain
Recall that many different cell types can all have receptors for a hormone
but each cell type can produce distinct responses.
In this case the control works to alter rate of secreting other items.
So T_3 exerts negative feedback on anterior pituitary's TSH secretion
T_4 exerts negative feedback on hypothalmus's secretion of TRH
The net result is to keep concentrations of T_3 and T_4 in a certain range

3

and so their effects are held in a range: homeostasis

Double hormone examples (Thyroid/Parathyroid and Pancreas)
Here two hormones have antagonistic effects relative to each other on a common feature.
Example: Blood calcium ion concentration homeostasis
     Fig. 45.10, human endocrine glands
     This involves hormones from thyroid and from parathyroid
     Fig. 45.1, (Campbell and Reece, 2002) blood calcium homeostasis
     Need to keep concentration of blood calcium about 10 mg/100 mL
     Fig. 45.11, calcium homeostasis (Campbell and Reece, 2005)
     Consider how to sense when beyond acceptable concentrations:
     Low blood [calcium ions]
          receptor in cells of parathyroid is empty as $[Ca^{+2}]$ is below its K_m for it
          parathyroid then releases parathyroid hormone (PTH)
          PTH is then bound by receptors at target organs and stimulates
               calcium release from bone
               calcium recovery in kidney
               calcium uptake in intestines and delivery to blood
               (this implies changes in membrane transport systems?)
          Note this is negative feedback in what way?
     high blood [calcium ions]
          receptor in cells of thyroid binds Ca^{+2}, as $[Ca^{+2}]$ is above K_m for it
          thyroid then releases calcitonin
          calcitonin is bound by receptors at target organs and stimulates
               calcium deposition in bones
               less calcium recovery in kidneys, so more left in urine...
          How is this negative feedback?
     See how these two negative feedback systems have antagonistic effects?
     Note the state of receptors for blood calcium ion
          if bound by thyroid, responds by hormone secretion
          if unbound at parathyroid, responds by hormone secretion
          So binding to a receptor or unbinding of a receptor can be used...
     note antagonistic effects results in homeostasis of this physiological trait
Example: Blood glucose concentration
     Fig. 45.6, human endocrine glands
     This involves the alpha and beta cells in the Islets of Langerhans in the pancreas
     Fig. 45.12, blood glucose homeostasis
     Concentration of glucose in blood has set point about 90 mg/100 mL
     Islets of Langerhans in pancreas
          alpha cells, secrete glucagon when activated
          beta cells, insulin secreted when activated
          each of these cell types must have receptors for blood glucose
     High [glucose]
          receptors on beta cells are filled, so insulin secreted into blood
          insulin receptors at target organs bind the insulin, this:

stimulates uptake of glucose by cells

and conversion in cells of glucose to glycogen

low [glucose]

receptors on alpha cells are empty, so glucagon secreted into blood

receptors for glucagon at target cells bind glucagon, this stimulates:

conversion of glycogen to glucose, and glucose put in blood

glucose uptake into cells is lower, left in blood

liver creates glucose (gluconeogenesis), sent to blood

(Note that hormone binding changes membrane transport activities.)

Do not confuse: glucose, glycogen, and glucagon

Be sure you can describe what the state of the receptors must be like

in the Islets cells to account for the secretion of these two hormones.

Notice that each hormone system is a negative feedback system

note antagonistic effects of these two hormones on same physiological feature

Not just glucose metabolism is regulated, but also lipids, amino acids, etc.

Fig. 47.20 (Purves et al., 1998) homoestasis_glucose.jpg

sugar, lipids, amino acids all connected

note intersections

Hypothalamus and Pituitary

Hypothalamus and posterior pituitary

Fig. 45.15, hypothalamus and posterior pituitary

both derived parts of the mammalian brain

note modified nerve cells in hypothalamus secrete items

that are stored in posterior pituitary

Can regulate posterior pituitary's secretion of these items into the blood

so here nervous tissue acts as an endocrine gland

also signals in nervous system can influence an endocrine gland's activity

Hypothalamus and anterior pituitary

Fig. 45.17, hypothalamus and anterior pituitary

anterior pituitary develops from the roof of mouth, epithelial not nervous tissue...

neurosecretory cells in hypothalamus secrete into capillary bed

note that this is the third portal system we have seen

two capillary beds in serries... What were the other two?

portal system carries hormones to capillary bed in anterior pituitary

these are often called "releasing hormones" or tropic hormones

tropic hormones influence secretions of other hormones

Anterior pituitary has receptors for hormones, and responds

So nervous system can influence activities of endocrine glands

Fig. 45.13, stress and adrenal glands

Common features of mammalian endocrine systems

endocrine glands (or can be just cells of an organ...)

items put into circulatory system

action at a distance from where released

movement through the circulatory system

only cells with receptors can respond, responses vary by cell type/location

use systems to monitor certain physiological features

Km of receptors may vary, responses due to receptor binding or unbinding

this influences endocrine secretions

homeostasis via negative feedback systems

single or double hormone systems, antagonistic effects

Similar systems occur in fungi, plants, and other multicellular species.

For mammals the circulatory system makes hormone movement fast (i.e. minutes)

other species may lack circulatory systems and hormone affects are slower

Objectives:

Be able to describe similarities and differences between hormones and: Pheromones, secondary messengers, and items used in synaptic or paracrine signaling. Be able to describe how hormonal regulation of an organism's functions makes use of cellular signal transduction systems. Be able to contrast features of hormonal control of responses between those involving changes in gene expression to those involving changes in the activity of existing proteins, and be able to name an example of each.

What is the difference between endocrine glands and exocrine glands? Be able to name examples of each as found in a mammal (figures 45.10 and 41.14 may be helpful). What is an example of a mammalian organ that is both an exocrine and an endocrine gland?

How does the mode of action of hydrophilic hormones generally differ from that of hydrophobic hormones? What are the general molecular classes of hormones found in mammals?

Be able to describe how negative feedback loops can help regulate the secretion of hormones and maintain a desired state. How is negative feedback seen in control of the thyroid and what other glands are involved (see fig. 45.18)? Be able to recognize how this does not involve the use of antagonistic hormones.

Be able to describe how homeostasis is achieved in the maintenance of blood calcium and blood glucose levels and the use of pairs of antagonistic hormones in these examples. Know the organs, hormones, and physiological effects involved. Consider the role of receptors, what items they must have binding sites for, and what the effect of their binding or not binding of those items should have on secretion of relevant hormones.

Be able to describe the relationship between the hypothalamus and anterior and posterior pituitary glands. In each case how do hormones get from the hypothalamus to either side of the pituitary? What is the functional role of the relationship between these endocrine glands? What is a portal system and what role does it play between these glands?

For review, see self-quiz questions #2-6 for chapter 45.

Needed overheads and items:

Fig. 11.5, common cell-siganling pathways in animals
Fig. 45.11, endocrine signaling pathway
Fig. 45.16, neurohormone signaling pathway
Fig. 45.18, neuroendocrine signaling pathway
Fig. 45.10, human endocrine glands
Table 45.1, list of endocrine glands and their hormones
Fig. 45.5, chemical signaling review
Fig. 45.8, diverse responses, same signals
Fig. 45.10, human endocrine glands
Fig. 45.12, (Campbell and Reece, 2002) catecholamine hormones
Fig. 45.7, (Campbell and Reece, 2002) T3 and T4 hormones
Fig. 29.13, (Zubay, 1983) thyroxine.jpg
Fig. 45.10, human endocrine glands
Fig. 29.10, (Zubay, 1983) insulin.jpg
Fig. 45.10, human endocrine glands
Fig. 45.13, (Campbell and Reece, 2002) steroid hormones
Table 45.1, list of endocrine glands and their hormones
Fig. 45.3, hormone types
Fig. 1.13, negative and positive feedback
Fig. 9.21, control of cellular respiration
Fig. 45.10, human endocrine glands
Fig. 45.9, negative feedback, thyroid hormones (Campbell and Reece, 2005)
Fig. 45.10, human endocrine glands
Fig. 45.1, (Campbell and Reece, 2002) blood calcium homeostasis
Fig. 45.11, calcium homeostasis (Campbell and Reece, 2005)
Fig. 45.10, human endocrine glands
Fig. 45.12, blood glucose homeostasis
Fig. 47.20 (Purves et al., 1998) homoestasis_glucose.jpg
Fig. 45.15, hypothalamus and posterior pituitary
Fig. 45.17, hypothalamus and anterior pituitary
Fig. 45.21, stress and adrenal glands

Handout:

Fig. 45.1, (Campbell and Reece, 2002) blood calcium homeostasis
Fig. 45.11, calcium homeostasis (Campbell and Reece, 2005)

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Hormones and the endocrine system. Chapter 45. Pages 975-996 and 207-210. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-Biology, 7th edition, Fig. 45.11. Benjamin Cummings Press. San Francisco, CA.

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Purves W.K., G.H. Orians, H.C. Heller, D. Sadava- 1998-Life: The Science of Biology. 5th edition. Fig. 47.20. Sinauer Associates, Inc. Sunderland, MA.

Zubay G- 1983-Biochemistry. Fig. 29.10, 29.13. Addison-Wesley Publishing Co. Reading, MA.

Related issues:

Studies of hormones involved in **glucose homeostasis** in animals, how errors in it lead to **diabetes**:, and attempts to understand and modify the workings of this system:

Couzin J- 2007-Tumor suppressor may also affect gestational diabetes- Science 318: (#5851, 11/2) 729

Dong M-Q., J.D. Venable, N. Au, T. Yu, S.K. Park, D. Cociorva, J.R. Johnson, A. Dillin, J.R. Yates III- 2007-Quantitative mass spectrometry identifies insulin signaling targets in *C. elegans*- Science 317: (#5838, 8/3) 660-663

Illies C., J. Gromada, R. Flume, B. Leibiger, J. Yu, K. Jubs, S-N. Yang, D.K. Barma, J.R. Falck, A. Saiardi, C.J. Barker, P-O. Berggren- 2007-Requirement of inositol pyrophosphates for full exocytotic capacity in pancreatic ß cells- Science 318: (#5854, 11/23) 1299-1302

Imai J., H. Katagirl, T. Yamada, Y. Ishigaki, T. Suzuki, H. Kudo, K. Uno, Y. Hasegawa, J. Gao, K. Kaneko, H. Ishihara, A. Niijima, M. Nakazato, T. Asano, Y. Minokoshi, Y. Oka- 2008-Regulation of pancreatic β cell mass by neuronal signals from the liver- 322: (#5905, 11/21) 1250-1254

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Nagamatsu S., M. Ohara-Imaízum- 2007-IP$_7$ debut in insulin release- Science 318: (#5854, 11/23) 1249-1250

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Taubes G- 2009-Prosperity's plague- Science (#5938, 7/7) 256-260

Yam P- 2003-Insulin from bone marrow- Scientific American 288; (#5) 33

Many hormones have multiple effects and roles. For instance, **glucagon** also has a role in processes other than sugar homeostasis:

Lin R.C., P.J. Snodgrass, D. Babier- 1982-Induction of urea cycle enzymes by glucagon and dexamethasome in monolayer cultures of adult rat hepatocytes- Journal of Biological Chemistry 257: (#9) 5061-5067

Here is a nice review of some of the hormones from the **pituitary**, amyloids.

Maji S.K., M.H. Perrin, M.R Sawaya, S. Jessberger, K. Vadodaria, R.A. Rissman, P.S. Singru, K.P.R. Nilsson, R. Simon, D. Schubert, D. Eisenberg, J. Rivier, P. Sawchenko, W. Vale, R. Riek- 2009-functional amyloids as natural storage of peptide hormones in pituitary secretory granules- Science 325: (#5938, 7/17) 328-332

Here are some studies of **calcium ion homeostasis** in animals.

Imura A., Y. Tsuji, M. Murata, R. Maeda, K. Kubota, A.Iwano, C. Obuse, K. Togashi, M. Tominaga, N. Kita, K-i. Tomiyama, J. Lijima, Y. Nabeshima, M. Fujioka, R. Asato, S. Tanaka, K. Kojima, J. Ito, K. Nozaki, N. Hashimoto, T. Ito, T. Nishio, T. Uchiyama, T. Fujimori, Y-i. Nabeshma- 2007-α-klotho as a regulator of calcium homeostasis- Science 316: (6/15) 1615-1618

Rosen C.J- 2003-Restoring aging bones- Scientific American 288: (#3) 70-77

For more on **endocrine systems** in other animals such as insects see:

Wigglesworth V.B- 1984-The endocrine system- Chapter 11, pgs. 179-184, in <u>Insect Physiology</u>. Chapman and Hall Publishers, London.

This article describes a new **gaseous hormone** (H_2S) that is used in animals.

Wang R- 2010-Toxic gas, life saver- Scientific American 302: (#3, March) 66-71

For a view of some **non-animal hormones** see:

Christianson M.L- 2000-Control of morphogenesis in byrophytes- Chapter 7, pgs. 199-224, in, Bryophyte biology. A.J. Shaw and B.F. Goffinet editors. Cambridge University Press, Cambridge.

Davies P.J- 1995-The plant hormones: Their nature, occurrence, and functions- Chapter 1, pgs 1-12 of Plant Hormones. Physiology, Biochemistry and Molecular Biology. 2nd edition. P.J. Davies editor. Academic Press.

Ferber D- 2005-Plant hormone's long-sought receptor found- Science 308: (5/27) 1240

Kende H- 2001-Hormone response mutants. A plethora of surprises- Plant Physiology 125: 81-84

Liu X., Y. Yue, Y. Nie, W. Li, W-H. Wu, L. Ma- 2007-A G protein-coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid- Science 315: (3/23) 1712-1716

BIO 107   2010
Day 9, Lecture 22, Title: Animal Reproduction
Text Readings: Campbell et al. (2008), chapter 46.

Topics to cover:

   Advantages of Asexual Reproduction
   Hermaphroditism and Plumbing Issues
   Mammalian System
     Male Reproductive System
     Female Reproductive System
     Contraceptive Methods
     Birth as a Positive Feedback System

Advantages of Asexual Reproduction
  Fig. 46.3, Handicaps of Sex
  Asexual reproduction produces more offspring per cycle than does sexual
    Do not need to find a mate, can be done by a single individual
      so good for dispersal to new areas, as no need to travel in pairs?
    Conserves genetic combination which if a good one is nice to keep.
  Examples of asexual reproduction in some animals
  Parthenogenesis: females create zygotes, not eggs, without males
    Fig. 46.4, parthenogenic lizard sexual behavior
    sexual behavior stimulates zygote production, no exchange of gametes here.
  Budding: sea anemone, splitting of adult into two
    Fig. 46.2, sea anemone
    Obviously no sexual recombination here.
Sexual reproduction.
  The text defines it as the fusion of gametes to create a zygote, but is perhaps too limited?
  Reproduction, new individuals created
    Asexual reproduction, the new individuals have nearly same genetic combination
      as the parents.
    Sexual reproduction, the new individuals have different genetic combinations
  Since meiosis creates new genetic combinations, it is part of the sexual act in animals?
    Though in animals it just makes gametes, it still is essential
    So sexual reproduction is more than just fusing of gametes, as text implies
  So meiosis might also be considered a sexual act?

Hermaphroditism and Plumbing Issues
Hermaphrodites
      An example is earthworm...
           both male and female, has potential to self mate
           But still has the option to cross mate if meets another individual
Plumbing of reproductive tract versus other tracts (see handout).
      Three systems all exit vertebrate animal body
           digestive, reproductive, excretory system
           how these meet up varies. Some species have three, two, or one opening
      cloaca, common exit for all three of the above systems.
           see cloaca in turtles, birds, and many other species
      urogenital_system.jpg, (Fig. 22, pg. L84, BIO 105, Fall Survival Manual, 2003)
      Fig. 16.72, (Hildebrand and Goslow, 2001) Cloaca and ducts, various groups
      Note in some cases sperm may move through kidney, or ureter.

Mammalian System
      Will use human system as basic model, works well for most mammals.
      Focus on production of gametes, and regulation of gonads by hormones
           This is another example of use of hormones to regulate things.

      Male Reproductive System
Spermatogenesis: production of sperm
      Fig. 46.11, human male reproductive anatomy
      Note location of: testis, epididymis, vas deferens, seminal vesicle, prostate gland,
           erectile tissue and prepus of penis.
      Fig. 46.12, spermatogenesis
           note error in book:
           Suggests just 4 sperm.
                In reality get many more than just four, as earlier several mitotic divisions
           Suggests separate early spermatids
                in reality have coenocytic spermatogenesis
                do not get cytokinesis until end of the process
      Fig. 20.29, (Alberts et al., 2002) spermatogenesis.jpg
           Sperm often share cytosol until the end of development, for a time multinucleate
           certain genetic consequences of this...
      Fig. 46.12, sperm
           Note: cytosol, mitochondria, centriole, flagella, acrosome, acrosomal vesicle
                and the nucleus. Note in figure the acrosomal is a vesicle in the cell.
           The nucleus and the centriole will be donated during fertilization.

Hormonal controls of sperm production
        Look at this first because it is simpler, and shows homeostasis, giving a stable state.
        Fig. 46.13, hormonal control of testes
        hypothalamus, secretes gonadotropic releasing hormones (GnRH)
                Stimulates release of Follicle stimulating hormone (FSH)
                        and luteinizing hormone (LH) from anterior pituitary
        FSH stimulates spermatogensis in testis
        LH promotes androgen (testosterone) production by testes
        testosterone acts to:
                promote sex development and secondary sex traits
                stimulate sperm production by certain cells in testis
                inhibit secretion by anterior pituitary and hypothalamus
        Also have inhibin made by Sertoli cells that exert negative feedback on anterior pituitary
        (i.e. negative feedback system...) (This is like what example we covered previously?)
        Due to this negative feedback have
                steady sperm production, and stable hormone concentrations in the blood serum.
        We will see a similar system in females, with a twist...

        Female Reproductive System
        Fig. 46.10, human female reproductive anatomy
        note:
                Ovary, follicles, oviduct, uterus, cervix, vagina.
oogenesis: production of egg (ovum)
        Fig. 46.12, oogenesis in ovary
        During ovum production there is interrupted meiosis, cells held this way for years
                This is a characteristic of animals only. (I have no idea why animals do this!)
                cells held as primary oocyte until follicle develops
                        arrested at prophase I of meiosis
                Then go to secondary oocyte stage until fertilized
                        here puts out one polar body, with chromosomes
                        and arrests at metaphase II of meiosis
                 (In BIO 108 we will cover meiosis and fertilization in more depth.)
                Finish egg formation only after start of fertilization!!
                        Only after fuses with a sperm is the egg actually formed.
                Polar bodies take away genetic material, note only one product of meiosis here
Ovarian cycle involves
        follicle formation, an immature follicle with a primary oocyte is activated
        when it matures see ovulation, the release of secondary oocyte
                 Note: the follicle does NOT release an egg, no egg formed yet.
        corpus luteum made from remains of follicle
                it secretes hormones and eventually degrades

menstrual cycle

       Fig. 46.13, hormonal control of testes

       Fig. 46.14, menstrual and ovarian cycle

       male system is stable, due to consistent negative feedback

       female system has fluctuating hormone concentrations in the blood serum

       But there are periods of relative stability when there is negative feedback used

              Shifts from negative feedback system to positive feedback system...

                    low concentrations of estrogen inhibit Ant. Pit. secretion of LH and FSH

                        this is negative feedback, (similar to what is seen in males?)

                    also see slow growth of the developing follicle, makes more estrogen

                    high concentrations of estrogen eventually made, these

                        stimulate GnRH secretion by hypothalamus

                        GnRH promotes LH and FSH , promoting estrogen production

                            this is positive feedback

                    high concentrations of both estrogen and progesterone inhibits

                        hypothalamus GnRH secretion

                        this is a return to negative feedback.

              Notice how before and after ovulation concentrations of LH and FSH are steady

                    at ovulation their concentrations spike.

                    So two times of negative feedback, one of positive feedback.

       Now relate above shifts to stages in ovarian and menstrual cycle

       ovarian cycle, involves changes in the follicle in the ovary through cycle

              follicular phase, development of a follicle and formation of secondary oocyte

              ovulation, brief, but a permanent change, so a result of positive feedback

                    secondary oocyte is released

              luteal phase, return to a more stable state

                    but note that follicle becomes a *corpus luteum* and

                      now makes both estrogen and more progesterone

       menstrual cycle, involves changes of the uterine lining

              menstrual flow phase, sheding of old lining

               proliferative phase, growth of new lining, induced by estrogen

              secretory phase, growth of capillaries in lining

                    induced by the combination of estrogen and progesterone

Consider this in more detail...

       5 hormones involved

              LH, FSH, estrogens, progesterone, GnRH

              Note: LH, FSH, GnRH also used by males.

       follicular phase

              LH and FSH at low concentrations

              immature follicle has receptors to FSH, not LH

                    here see control of response by receptor presence/absence

              FSH stimulates follicle growth,

                    As gets larger, more estrogen secreted by it

                    low [estrogen] inhibits FSH and LH secretion by Ant. Pit.

                    high [estrogen] stimulates GnRH by hypothalamus

so spike in LH and FSH occurs

note change in response with change in concentration here

estrogen also increases pituitary sensitivity to GnRH

spike of LH and FSH concentrations

follicle now developed enough to have functional LH receptors

LH promotes estrogen secretion, which stimulates LH secretion,

positive feedback system, inherently unstable, promotes change

results in ovulation

Note occurs a bit after peak of LH and FSH concentrations

Luteal phase

now have follicle tissue in ovary converted into a *corpus luteum*

LH stimulates it to secrete estrogen and more progesterone

these give negative feedback on hypothalamus, so less GnRH secreted

So less LH and FSH released by anterior pituitary

so a return to a fairly stable situation

corpus luteum slowly degrades as LH drops, see slow drop in hormone production

progesterone promotes thickening lining of endrometrium

loss sets stage for menstrual flow

Implantation, in humans about 7 days after fertilization

By this point the zygote has developed into a small embryo, a blastocyst

Fig. 46.15, implantation, postfertilization

young embryo secretes human choronic gonadotropin (HCG)

acts like LH to maintain *corpus luteum*

so it does not degrade, and progesterone still made, and no menstrual flow

Contraceptive Methods

Fig. 46.20, contraceptive methods

male methods

condom use, vasectomy, but *coitus intrruptus* and abstinence....?

(?Why are these last two only applied to males?)

female methods

birth control pill

progesterone acts to block LH release

inhibits ovulation

estrogen blocks GnRH release, and blocks release of FSH

inhibits follicle development

reversible and irreversible methods

Physical ones: Tubal ligation and vasetomy

condom, diaphragm, cervical cap, IUD

Behavioral controls:

coitus interruptus, abstinence (?Again, why is this only the guy?)

Drugs: birth control pill (to prevent ovulation)

spermicides

morning after pill, etc...

Birth as a Positive Feedback System
Positive feedback leading to birth

        Fig. 46.18, hormonal control of labor

        note

                estrogen from Mom influences uterus

                        induces oxytocin receptors to be deployed

                oxytocin is from fetus and Mom's posterior pituitary

                Note: This is OXYTOCIN, not the pain killer with the similar name...

                Reception of oxytocin stimulates prostaglandin secretion by placenta

                        so placenta is an endocrine gland

                contractions stimulate more

                        oxytocin and prostaglandin secretions

                leads to labor, the result of a positive feedback loop

Traversing the human birth canal by an infant with a large head.

        (Rosenberg and Trevathan, 2001) human_birth.jpg

                Birth canal in humans is widest side to side, and narrower front to back at start. But towards outer end it is widest front to back and narrower side to side. This means that the head must come out at a 90 degree angle relative to the shoulders of the child. The infant has to twist as it comes out.

                Average female pelvic opening is 13 cm at the largest diameter and 10 cm at smallest. Average human infant head is about 10 cm across, with shoulders 12 cm across.

If time: Discuss what can cross the placenta?

Objectives:

Be able to compare the costs and benefits of sexual versus asexual reproduction.

What is a cloaca and what major organ systems normally feed into it? What is an example of an animal species that has a cloaca? Know the basic structures associated with the mammalian reproductive system, both males and females.

The sequence of steps in the menstrual and ovarian cycle, and how these relate to changes in concentrations of hormones, are worth knowing (Fig. 46.14 is a good summary). What hormones are involved and what are their effects at each stage of these cycles? What roles do the follicle and *corpus luteum* play, and when do they play them? Contrast the effects of key hormones in the mammalian female with the effects of similar hormones in the mammalian male: For instance, be able to contrast the role of FSH and LH in control of male and female reproductive organs, and how their secretions are controlled. What are the differences between mammalian oogenesis and spermatogenesis?

The induction of labor is a good example of a positive feedback system. Be able to describe in what way this is so, and in what way ovulation is also an example of a positive feedback system. Be able to describe examples that illustrate negative feedback seen in both male and female mammalian reproductive systems.

Be able to describe at least two methods of contraception used by human males and two used by females. What is the mode of action of each?

For review see self-quiz questions #3, 6 and 8 at the end of the chapter.

Needed overheads and items:
      Fig. 46.3, Handicaps of Sex
      Fig. 46.4, parthenogenic lizard sexual behavior
      Fig. 46.2, sea anenome
      urogenital_system.jpg, (Fig. 22, pg. L84, BIO 105, Fall Survival Manual, 2003)
      Fig. 16.72, (Hildebrand and Goslow, 2001) Cloaca and ducts, various groups
      Fig. 46.11, human male reproductive anatomy
      Fig. 46.12, spermatogenesis
      Fig. 20.29, (Alberts et al., 2002) spermatogenesis.jpg
      Fig. 46.12, sperm
      Fig. 46.13, hormonal control of testes
      Fig. 46.10, human female reproductive anatomy
      Fig. 46.12, oogenesis
      Fig. 46.13, hormonal control of testes
      Fig. 46.14, menstrual and ovarian cycle
      Fig. 46.15, implantation, postfertilization
      Fig. 46.20, contraceptive methods
      Fig. 46.18, hormonal control of labor
      (Rosenberg and Trevathan, 2001) human_birth.jpg

handout:
      urogenital_system.jpg, (Fig. 22, pg. L84, BIO 105, Fall Survival Manual, 2003)
      Fig. 16.72, (Hildebrand and Goslow, 2001) Cloaca and ducts, various groups

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P.Walter- 2002-<u>Molecular Biology of the Cell</u>. 4th edition. Fig. 20.29. Garland Science Publishers. N.Y., N.Y.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Animal reproduction. Chapter 46. Pages 997-1020. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Rosenberg K.R., W.R. Trevathan- 2001-The evolution of human birth- Scientific American 285: (#5) 72-77

Hildebrand M., G. Goslow- 2001-Reproductive system and urogenital ducts. Chapter 16, Figure 16.72, in <u>Analysis of Vertebrate Structure</u>. 5th edition. John Wiley & Sons, Inc. N.Y., N.Y.

Introductory Biology 105: Survival Manual- 2003-Fig. 22, pg. L84. Cornell University. Ithaca, N.Y.

Related issues:

It used to be thought that the condensed chromosomes of the **oocyte**, being arrested in the middle of meiosis, did not express any genes. It turns out that some **gene expression** does occur for in the oocyte. Here is an example where failure to express one gene properly in oocytes results in extremely rapid development of the follicles leading to early menopause.

Marx J- 2008-Aging of the ovary linked to PTEN pathway- Science 319: (#5863, 2/1) 558-559

Reddy P., L. Liu, D. Adhikari, K. Jagarlamndi, S. Rajareddy, Y. Shen, C. Du, W. Tang, T. Hämäläinen, S.L. Peng, Z-J. Lan, A.J. Coonex, I. Huntaniemi, K. Liu- 2008-Oocyte-specific deletion of *pten* causes premature activation of the primordial follicle pool- Science 319: (#5863, 2/1) 611-613

For more on **oogenesis** and **ovulation** in mammals see:

Duggavathi R., R.D. Murphy- 2009-Ovulation signals- Science 324: (#5929, 5/15) 890-891

Fan H-Y., Z. Liu, M. Shimada, E. Sterpeck, P.F. Johnson, S.M. Hedrick, J.S. Richards- 2009-MAPK3/1 (ERK1/2) in ovarian granulosa cells are essential for female fertility- Science 324; (#5929, 5/15) 938-941

Fan W., K.G. Waymire, N. Narula, P. Li, C. Rocher, P.E. Coskun, M.A. Vannan, J. Narula, G.R. MacGregor, D.C. Wallace- 2008-A mouse model of mitochondrial disease reveals germline selection against severe mtDNA mutations- Science 319: (#5865, 2/15) 958-962

Normile D- 2009-Study suggests a renewable source of eggs and stirs more controversy- Science 324: (#5925, 4/17) 320

Stitzel M.L., G. Seydoux- 2007-Regulation fo the oocyte-to-zygote transition- Science 316: (4/20) 407-408

Vogel G- 2005-Controversial study finds an unexpected source of oocytes- Science 309: (7/29) 678-679

Remember that not all animals share our pattern of sexual reproduction. For instance, many species are **hermaphrodites**. Here is a study looking at how hermaphroditism might be produced.

Baldi C., S. Cho, R.E. Ellis- 2009-Mutations in two independent pathways are sufficient to create hermaphroditic nematodes- Science 326: (#5955, 11/13) 1002-1005

Harada Y., Y. Takagaki, M. Sunagawa, T. Saito, L. Yamada, H. Taniguchi, E. Shaguchi, H. Sawada- 2008-Mechanism of self-sterility in a hermaphroditic chordate- Science 320: (#5875, 4/25) 548-550

Conditions in the mammalian **womb** are complex, they can change and influence fetal development. For instance, whether the mother catches the flu can matter. It has also been found that various molecules, and even cells, can cross the **placenta** and move between embryos or the mother. Fetal cells have been found in the mother, and visa versa.

Castelvecchi D- 2009-The placenta- Scientific American 301: (#3, Sept.) 83

Choi C.Q- 2005-Baby to brain. Therapy clues from fetal cells that enter Mom's brain- Scientific American 293: (#5, Nov.) 22-23

Isoda T., A.M. Ford, D. Tornizawa, F.W. van Delft, D.G. deCastro, N. Mitsuiki, J. Score, T. Taki, T. Morio, M. Takagi, H. Saji, M. Greaves, S.M. Zutoni- 2009-Immunologically silent cancer clone transmission from mother to offspring- Proceedings of the National Academy of Science, USA 106: (#42, 10/20) 17882-17885

Leslie M- 2008-Fetal immune system hushes attacks on maternal cells- Science 322: (#5907, 12/5) 1450-1451

Melton L- 2000-Womb wars: New evidence that a mothers's and father's "imprinted genes" battle to determine a baby's size- Scientific American 283: (#4) 24-26

Patterson P.H- 2007-Maternal effects on schizophrenia risk- Science 318: (#5850, 10/26) 576-577

Soares C- 2007-All in the family- Scientific American 297: (#1, July) 30

Stix G- 2007-Selfless giving. Mom's brain chemical affects embryonic development- Scientific American 296: (#4, April) 20-22

Tyrio R., R. Cossart, I. Khalilov, M. Minlebaev, C.A. Hübner, A. Represa, Y. Ben-Ari, R. Khazipov- 2006-Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery- Science 314: (12/15) 1788-1792

In vitro **fertilization** is becoming more commonly used by humans. Here is a recent article discussing the use of oocytes that had been frozen for a long time for IVF:

Leslie M- 2007-Melting opposition to frozen eggs- Science 316: (4/20) 388-389

For more on **spermatogenesis** and **male organs** in mammals see:

Brinster R.L- 2007-Male germline stem cells: From mice to men- Science 316: (4/20) 404-405

DiNardo S., R.E. Braun- 2007-Home for the precious few- Science 317: (#5845, 9/21) 1696-1697

Goldstein I- 2000-Male sexual circuitry- Scientific American 283: (#2) 70-75

Sada A., A. Suzuki, H. Suzuki, Y. Saga- 2009-The RNA-binding protein spermatogonial stem cells- Science 325: (#5946, 9/11) 1394-1398

Yoshida S., M. Sukeno, Y-I. Nabeshima- 2007-A vasculature-associated niche for undifferentiated spermatogonia in the mouse testis- Science 317: (#5845, 9/21) 1722-1726

For more on mating of non-human animals. Here are articles on sexual conflicts involved in **mate choice**, **insect reproduction**, another on **germ line cells**., **asexual reproducers**, and a report on the **longest sperm** ever measured!

Dean R., M.B. Bonsall, T. Pizzari- 2007-Aging and sexual conflict- 316: (4/20) 383-384

Kimble J., D.C. Page- 2007-The mysteries of sexual identity: The germ cell's perspective- Science 316: (4/20) 400-401

Maggioncalda A.N., R.M. Sapolsky- 2002-Disturbing behaviors of the orangutan- Scientific American 286: (#6) 60-65

Mealey L- 2000-Mating systems. Chapter 7, pgs. 143-172. In, Sex Differences. Developmental and Evolutionary Strategies. Academic Press, San Diego, Ca.

Pitnick S., G.S. Spicer, T.A. Markow- 1995-How long is a giant sperm- Nature 375: (#6527) 109

Pouchkina-Stantcheva N.N., B.M. McGee, C. Boschetti, D. Tolleter, S. Chakrabortee, A.V. Popova, F. Meersman, D. Macherel, D.K. Hincha, A. Tunnacliffe- 2007- Functional divergence of former alleles in an ancient asexual invertebrate- Science 318: (#5848, 10/12) 268-271

Wigglesworth V.B- 1984-Reproduction- Chapter 8, pgs. 132-144, in Insect Physiology. Chapman and Hall Publishers, London, U.K..

Estrogen and **progesterone** do more than just regulate the ovarian cycle. For instance, they influence changes in the **maternal brain**. Also, long term exposure to progesterone has been suggested to promote **breast cancer** leading to treatments being developed involve the use of inhibitors of the progesterone receptors.

Kinsley C.H., K.G. Lambert- 2006-The maternal brain- Scientific American 294: (#1, Jan.) 72-79

Marx J- 2006-Squelching progesterone's signal may prevent breast cancer- Science 314: (12/1) 1370

In some species the **sperm** donates a lot more to the zygote than it does in animals. Here is one example.

Grossniklaus U- 2009-Paternal patterning cue- Science 323: (#5920, 3/13) 1439-1440

BIO 107   2010
Lecture 23, Title: Transport II
Text Readings: Campbell et al. (2008) Chapter 36.

Topics to cover:

> **Long Distance Transport in Plants**
> **Water Potential and Plant Compartments**
> **Xylem: Two systems**
> > **Root Pressure**
> > **Evapotranspiration**
>
> **Phloem:**
> > **Apoplastic Loading**
>
> **Summary**

Long Distance Transport in Plants
Fig. 36.2, water transport whole plant
> water vs. oxygen vs. carbon dioxide in terms of diffusion
> > plant organism is too large for diffusion only, so must move items
> > carbon dioxide enters by diffusion, but in gaseous form
> > > letting it in allows water vapor to exit
>
> note roots vs. leaves
> > > roots: do respiration, need oxygen from soil, has lots of water
> > > > so gas exchange here is for mainly for oxygen gas
> > > > oxygen gas is used for respiration of sugars
> > > > for ATP whose hydrolysis is used to power growth
> > >
> > > leaves: do P.S., need carbon dioxide, lose water release oxygen gas
> > > > so gas exchange here is mainly for carbon dioxide.
>
> So to allow gas exchange at leaves must move water to them to keep them moist.
> > to get sugars to roots must move them from leaves to roots
>
> Both of these are beyond the range at which diffusion will operate, so need bulk flow

Vascular tissue is the plant's circulatory system, not found in all plants, just vascular plants.
> xylem: one way, soil to leaves, by two distinct mechanisms as we will see
> phloem: potentially several directions
> > from region that loads phloem (source) to that which uses item from it (sink)
>
> note potential for water circulating in loop, but most leaves plant

Water Potential and Plant Compartments
> plasmolysis and turgor, osmosis
> > Fig. 36.9, water relations in plant cells
> > recall that uptake of solutes, results in turgid cells.
> > what if put a straw in it? Water pressure would push solution up the straw?

Water Potential components WP = P + S
  has pressure and solute concentration components.
  Water potential is proportional to the free energy of water,
    but different units. Pressure, not Kcal/mole.
  This turgid cell's water potential is the same as the surrounding solution
    but it has negative solute potential balanced by positive pressure
    the sum of these gives the same water potential as the water surrounding it
  Thus even at the same water potential can create pressure.
    So this is a means to generate pressure gradients
  Pressure or tension, gradient will moves water by bulk flow
  solute concentration inversely related to water concentration
    (see water_conc.jpg, on water and sucrose concentration, Weast, 1975)
    it can generate water potential, and also cause water to move by osmosis
moving solutes vs. moving water
  water crosses membranes easily
  solutes do not, so move solutes and let water follow passively, osmosis
differences in water potential, found vs. made
  Humans create differences in water potential
    heart pumping creates pressure gradient, but costs energy
  Clothes dry because of a gradient in free energy of water in two places
  Can pump solutes at cell level to create pressure differences, costs energy
  Plants encounter a free gradient from soil to air, which costs nothing to the plant.
What happens in xylem and phloem is that water potential gradients are used or made
    to induce bulk flow of water down pressure/tension gradients
    this achieves long distance transport in the organism
Compartments in the plant
  Instead of turgor pressure in a cell, will now generate pressure in a tissue
    or hold tension in a tissue, and use that to drive bulk flow
  What compartments are used?
    apoplast vs. symplast
    Fig. 36.11, compartments in tissues
    Fig. 6.28, plant cell walls and PD
  apoplastic spaces: nonliving, cell walls, air spaces, etc
    by cell-cell connections, and barriers in cell walls, can make apoplastic areas
    able to hold pressure or tension (our cells can not do this...)
    but to do this have to make barriers to hold the pressure or the solutes
  symplastic spaces: all living, cytoplasm, nuclei, protoplasmic areas...
    connect via plasmodesmata so all cytosols can be connected
    Use plasma membranes, to hold solutes, cell walls to tolerate pressures
  In the vascular tissue of plants we will see use of apoplastic regions and symplastic regions
    these regions can be separated from other such regions
    this produces areas with distinct components of water potential
    so plant can use this to generate changes in pressure in distinct places...

Xylem: Two systems

  Xylem cells are dead at maturity, just cell walls

  Connect to make long vessels up to a meter long in some cases

    cross linked by pores through cell walls with other vessels

  The xylem has two mechanisms (two systems) that it uses to drive bulk flow

    One used during wet atmospheric conditions (root pressure)

    Other used during dry atmospheric conditions (evapotranspiration)

    Both involve differences in pressure that drive bulk flow

  The same plant can use both systems, but NOT at the same time

    since the atmosphere can not be both wet and dry at the same time.

Root Pressure

  Used when air is wet, can push xylem solution up stem

  Powered by the uptake of solutes, costs energy as must power cotransport systems

    need transport systems in membrane to move ions from soil solution into cells

  Then dump ions into an apoplastic compartment,

    this alters the solute potential there, and draws in water by osmosis,

    generating pressure just like in a turgid cell

  These ions are put into the stele of the root, if allowed to leak out the solute potential

    will degrade and little pressure will result

    the plasma membranes of the cells are not leaky to ions,

    but the cell walls are typically open and ions could leak through here.

    So the symplast in the root is a barrier, only passed by ion transport sytems

      so movement into/out of symplast is controllable, no leakage here.

    But the apoplastic space, the cell walls, are leaky, ions put into the xylem in

      the stele could diffuse down their concentration gradient through cell

      walls unless a barrier to their flow is put in place.

  That barrier in the apoplast occurs in cell walls of the endodermis

  Casparian strip, used to seal off cell walls to separate apoplast of stele from outer cortex

    Fig. 36.12, movement of items into root

    This is a waxy layer in just sides of cells, water can not pass,

      so ions dissolved in the water in the cell walls can not pass

    note the apoplastic path vs symplastic path for water flow

    note location and role of Casparian strip

    CasparianStrip.jpeg (Mauseth, 1998, fig. 7.13)

  pressure generation drives bulk flow

    So living cells spend ATP to pump ions into them

      pass the ions through the symplast, and dump the ions into the stele

    That increases the concentration of solutes in the stele, where the xylem is

    This pulls in water by osmosis, and generates pressure

      just like a turgid cell, are at the same water potential as soil solution

      but are balancing a negative solute potential with a positive pressure

The solutes are kept from leaking out by the Casparian strip in the
cell walls of the endodermal cells around the stele.
So solutes kept in the stele, pressure is made by osmosis
That pressure pushes up through the xylem vessels to the stem, and leaves.
just like cell turgor just larger compartment
can make enough pressure to push xylem sap up about 10 meters
Results in guttation
Fig. 36.13, guttation
So this is xylem sap that flows, water, salts, hormones, etc.
sent from roots to leaves.
Leaves need no water as are wet, this delivers mineral nutrients to leaves
(Students might consider how bulk flow seen in this root pressure system
compares to that seen in bird salt glands, or insect malphigian tubules?)
when is this used?
rainy days, costs energy so is a last resort
requires transport of ions across living cells into apoplastic space
why used if it costs energy?   Leaves would be nutrient starved otherwise.
So a one-way transport system.

Evapotranspiration
This is the second mechanism that is used in the xylem of vascular plants
Used on days when air surrounding the leaves is dry.
Dry air has very negative water potential, so water evaporates and tension is made
that tension moves through the plant to the soil
Fig. 36.15, ascent of water in a tree
Note the tension from the air to the soil solution
Recall pressure units: 1 MPa = 10 bars = about 10 atmospheres
So an exisiting gradient, no need to make one, so is cheap for plant to use
all the plant has to do is make a conduit, open it, and water will flow
passive evaporation from the leaves
evapotranspiration influenced by leaf structures
two gases are of interest at the leaves, CO_2 and water vapor
Fig. 36.14, leaf evaporation
note: waxy cuticle, over most of surface, a barrier to water loss and CO_2 entry
guard cells, can open/close pores and regulate rate of gas exchange
this is critical to prevent cells in leaf from drying out
vascular bundle and xylem, water delivered here
Other than the costs of regulation and building the structures this is a passive
process that costs the plant little,
driven by water cohesive/adhesive properties
water is cohesive, through H-bonds,
so tension is transmitted in the xylem down stem to roots, and out to soil water
This generates a pressure gradient that drives bulk flow...

Regulation of evaporation, leaf must detect water potential of leaf
      cuticle on leaves
            Fig. 36.14, note cuticle
      guard cells, two of them make up a stoma with a pore between
      many of these on the leaf surface, to open the pore need to swell the guard cells
            Fig. 36.17, K^+ role in guard cell function
            Fig. 36.16, stomata
            H^+ pumping ATPase produces membrane potential difference
            K^+ ion channel opens, and membrane potential pulls in K^+
            Rise in $[K^+]$ pulls water in by osmosis, cell swells, pore opens
            Note: K^+ here is moving passively UP its concentration gradient
      If not regulated, then plant wilts
            Fig. 36.10   wilted and turgid plants
            leaf water potential too negative, stomatal pores close
                to try to keep water in the plant, used as hydrostatic skeleton?
                but this also prevents CO_2 entry, so photosynthesis shuts down
                wilted plants do little photosynthesis...
            If concentration of CO_2 in leaf is high, stomatal pores close
                why lose water for no reason?
                only keep the pores open to let CO_2 in...
    Role of the Casparian strip in transpiration
            Fig. 36.12, root structures
      water can not pass strip, neither can items dissolved in water cross strip
      but the stele is under tension, and that tension pulls water across cell membranes
            That transmits the tension through the symplast
            then out of symplast to apoplast of root and so out to soil
      amount of flow on a sunny day, 100s of liters per hour per large tree
      if toxic items are dissolved in water, if bacteria are present, or fungal spore
            these would get a free ride into the plant and up to the leaves?
      To prevent this use the Casparian strip to force items to cross a membrane
      strip blocks items from non-selective entry, must pass a membrane in a cell
            water passes easily,
            but ions' and other solutes' entry is controlled
                by membrane transport systems
            and bacteria and fungal spores can not enter
            so toxic items not given a free ride to leaves, microbes stopped...
      (Relate this to the way animals handle their toxins at the kidney?)
Note: Root pressure and transpiration-cohesion-tension systems use same structures
    costs are different,
    but both allow a plant to get items from roots to leaves by bulk flow

Phloem:

    At least two mechanisms of loading items into phloem, will cover just one...
        (The other major mechanism is symplastic loading...)

Apoplastic Loading

At leaves make solutes like sugars, amino acids.
Move these to phloem and put them into these cells, we call this phloem loading
    This creates a high solute concentration in these cells
    pulls in water by osmosis, which generates pressure
So once again, we are playing with the components of water potential
    at the same water potential moving ions, allows osmosis to generate pressure
Pressure gradient through phloem, so phloem sap flows down pressure gradient,
Note that the loading costs energy
Fig. 36.19, phloem loading
Note error in this figure., shows two mechanism,
    only one of these works at a time.
        (cross out symplastic loading pathway from image)
Active transport systems load sucrose at sources
    proton pumping ATPase, and proton/sucrose symport systems all involved
passive transport systems unload at sinks
So flow is from source to sink.
A sink can be any region using the sugars and other organic matter
    A root, a growing leaf, a swelling fruit...
    Fig. 36.20
        note unloading, need sucrose premease transport system, passive
Pressure differences produce bulk flow
        pressure gradients of 1-10 Atm seen in plant phloem
        compare to human blood pressure (1 atm = 760 mm Hg)
Note that phloem cells are symplastically connected, through modified plasmodesmata
    Thus solutes put in phloem are surrounded by cell membranes,
        this hold solutes, and so pressure, in these cells
How to get at phloem sap to study it?
    Such high pressure that to cut it open results in sap jetting out.
    Fig. 36.21, aphid feeding
    pressure is so great the aphids do not suck out sap, it flows through them
    useful system for sampling phloem sap for study...

Summary

Fig. 28.23, (Raven etal., 1992) water flow in vascular tissue
    Note that some water cycles through plant, but the vast majority is evaporated at leaves
    These two systems give a means of circulating materials in plant organism
        without heart or organ pumps, all at cellular level

Water moves due to
       pressure gradients
       concentration gradients
           but always to lower energy level
       over short distances water may move by diffusion,
           across membranes we call it osmosis,
           but movement due to long distance pressure gradients is called bulk flow...
Life can move salts, create concentration gradients which results in pressure gradients
           this is the essence of flow via root pressure and in the phloem
       These gradients can occur in living cell compartments (symplastic)
           or in intracellular spaces (apoplastic)
Or life can create pressure gradients in an isomotic environment (heart pumping)
Allows movement of items over long distances, allows for large multicelled existence...
       After fast, pressure-driven, flow, then have local exchange into/out of cells.
So a different set of solutions to some of the same problems seen in other multicellular organisms
       (i.e. animals).

Functional need in plant:	Equivalent system(s) in animals:
Gas exchange (need moist leaves)	Respiratory
Move items internally (past diffusion limits)	Circulatory
Keep internal fluids distinct (waxy cuticle, stomates, Casparian strip...)	Osmoregulation, excretory
Long distance signaling (hormones and action potentials)	Endorcrine, nervous

Objectives:

What is water potential, and how does water movement relate to water potential gradients? What factors influence the water potential and which of these do plants actively manipulate? If you understand the concept of water potential you should be able to describe the flow through the xylem and through the phloem, or even through the human circulatory system, in terms of the factors that contribute to water potential gradients.

Be able to describe the flow of water from the soil through the roots, up the stem, and out of the leaves of a plant. What structures and cell types are used by vascular plants to allow, or to regulate, the flow of water through their bodies?

How is root pressure generated? Does creating root pressure cost the plant metabolic energy; if so what is this energy used to do? Be able to identify the molecular events that connect metabolic energy use by the plant to a final pressure in the root. In what cells and region of the root is root pressure created? Under what conditions will root pressure be used, and what items does it typically move? What components of water potential does the plant modify to produce root pressure? Be able to describe the structure of the Casparian strip and its role in both root pressure and during transpirational flow.

Describe how the transpiration of water results in the bulk flow of water from the soil to the leaves. Does this flow cost the plant metabolic energy; if so how? What structures in the leaves of vascular plants allow the plant to control transpiration? What functions do guard cells and cuticle play in the control of transpiration, and how do they do it? How else could a plant modify its structure to alter its rate of water loss?

How is bulk water flow generated in phloem? What cells are involved in apoplastic phloem loading, and how is that loading achieved? What items are typically moved through the phloem? What areas of a plant are considered to be "sources" and what areas of a plant can be considered to be "sinks"?

For review see self-quiz questions #2-9 and 11 of this chapter.

Needed overheads and items:

Fig. 36.2, plant transport
Fig. 36.9, cell water relations
overhead on water and sucrose concentration (Weast, 1975; water_conc.jpg)
Fig. 36.11, cell compartments in plants
Fig. 6.28, plant cell wall
Fig. 36.12, root lateral transport
CasparianStrip.jpeg (Mauseth, 1998, fig. 7.13)
Fig. 36.13, guttation
Fig. 36.15, ascent of water up a tree
Fig. 36.14, leaf transpirational pull
Fig. 36.17, stomata and potassium
Fig. 36.16, stomata, light micrographs
Fig. 36.10, wilted and turgid plants
Fig. 36.12, root lateral transport
Fig. 36.19, phloem loading
Fig. 36.20, sieve tube pressure gradients
Fig. 36.21, aphid tapping phloem sap
Fig. 28.23, (Raven etal., 1992), Pressure flow mechanisms

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Resource acquisition and transport in vascular plants. Chapter 36. Pages 764-784. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Mauseth J.D- 1998-<u>Botany: An introduction to Plant Biology</u>. Jones and Bartlett Publishers. Sudbury, MA. Fig. 7.13.

Raven P.H., R.F. Evert, S.E. Eichhorn- 1992- <u>Biology of Plants</u>. 5th Edition. Fig. 28.23. Worth Publishers, N.Y., N.Y.

Weast R.C. (editor)- 1975-<u>Handbook of Chemistry and Physics</u>- 56th edition. Pg. D-261. CRC Press. Cleveland, Ohio.

Related issues:

Issues relating to the **xylem**:
For more on the transpiration and the physics of xylem sap flow see:

Nobel P.S- 1974-<u>Introduction of Biophysical Plant Physiology</u>. W.H. Freeman and
Company. San Francisco, CA.

The tension in xylem sap can be so great that air bubbles form, leading to **cavitation** of the sap. How the plant fixes these cavitated blocks in its transpiration is an area of interest.

Canny M.J- 1998-Applications of the compensating pressure theory of water transport-
American Journal of Botany 85: (#7) 897-909

Cochard H., C. Bodet, T. Améglio, P. Cruiziat- 2000-Cryo-scanning electron microscropy
observations of vessel content during transpiration in walnut petioles. Facts or
artifacts?- Pland Physiology 124: 1191-1202

Holbrook N.M., M.A. Zwieniecki- 1999-Embolism repair and xylem tension: Do we need
a miracle?- Plant Physiology   120: (#1) 7-10

Holbrook N.M, E.T. Ahrens, M.J. Burns, M.A. Zwieniecki- 2001-*In vivo* observation of
cavitation and embolism repair under magnetic resonance imaging- Plant
Physiology 126: 27-31

Zwieniecki M.A., N.M. Holbrook- 2000-Bordered pit structure and vessel wall surface
properties. Implications for embolism repair- Plant Physiology 123: 1015-1020

When xylem is cut, the plant must plug the holes. This is done by growing extensions, **tyloses**, from the surrounding cells into the xylem vessels.

Cochard H., L. Coll, X. LeRoux, T. Améglio- 2002-Unraveling the effects of plant
hydraulics on stomatal closure during water stress in walnuts- Plant Physiology
128: 282-290

Sun Q., T.L. Rost, M.A. Matthews- 2006-Pruning-induced tylose development in stems
of current-year shoots of *Vitis vinifera* (Vitaceae)- American Journal of Botany
93: (#11) 1567-1576

Here is a review about the **endodermis** and its **Casparian strip**. Features of the endodermis are found in other organs than just the roots.

Lersten N.R- 1997-Occurrence of endodermis with a casparian strip in stem and leaf- The
Botanical Review 63: (#3) 265-272

The xylem carries more than water and minerals. **Plant hormones** are carried in xylem sap, and sap pH acts as a signal to the leaves. Also **pathogens** have been suggested to move through xylem vessels, implying a need for plants to have defenses against pathogens in their vascular system.

Else M.A., A.E. Tiekstra, S.J. Croker, W.J. Davies, M.B. Jackson- 1996-Stomatal closure in flooded tomato plants involves abscisic acid and a chemically unidentified anti-transpirant in xylem sap- Plant Physiology 112: 239-247

Thorne E.T., B.M. Young, G.M. Young, J.F. Stevenson, J.M. Labavitch, M.A. Matthews, T.L. Rost- 2006-The structure of xylem vessels in grape fruit (Vitaceae) and a possible passive mechanism for the systematic spread of bacterial disease- American Journal of Botany 93: (#4) 497-504

Wilkinson S., J.E. Corlett, L. Oger, W.J. Davies- 1998-Effects of xylem pH on transpiration from wild-type and flacca tomato leaves- Plant Physiology 117: 703-709

Issues relating to the **phloem**:

For more on **phloem unloading** and **phloem loading** mechanisms see:

Kempers R., A. Ammerlann, A.J.E. van Bel- 1998-Symplastic constriction and ultrastructural features of the sieve element/companion cell complex in the transport phloem of apoplasmically and symplasmically phloem-loading species- Plant Physiology 116: 271-278

Patrick J.W- 1997-Phloem unloading: Sieve element unloading and post-sieve element transport- Annual Review of Plant Physiology and Plant Molecular Biology 48: 191-222

Transition of developing leaves from **sinks** to **sources** of organic matter:

Evert R.F., W.A. Russin, A.M. Bosabalidis- 1996-Anatomical and ultrastructural changes associated with sink-to-source transition in developing maize leaves- International Journal of Plant Science 157: (33) 247-261

Here is a classical paper measuring the **phloem pressure**.

Hammel H.T- 1968-Measurement of turgor pressure and its gradient in the phloem of oak- Plant Physiology 43: 1042-1048

There are reports of **small RNA**s moving through the **phloem**, making it a means for gene-coding information to move through out the body of the plant!

Dunoyer P., G. Schott, C. Himber, D. Meyer, A. Takeda, J.C. Carrington, O. Voinnet- 2010- Small RNA duplexes function as mobile silencing signals between plant cells- Science 328: (#5980, 5/14) 912-916

Martienssen R- 2010-Small RNA makes it mobile- Science 328: (#5980, 5/14) 834-835

Molnar A., C.W. Melnyk, A. Bassett, T.J. Hardcastle, R. Dunn, D.C. Baukombe- 2010-Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells- Science 328: (#5980, 5/14) 872-875

Tamaki S., S. Matsuo, H.L. Wong, S. Yokoi, K. Shimamoto- 2007-Hd3a is a mobile flowering signal in rice- Science 316: (5/18) 1033-1036

The phloem moves many other items as well. Including chemical signals to alter gene expression to promote **viral defenses**. Other signals relate to sensing of **day length** that alters **flowering**.

Corbesier L., C. Vincent, S. Jang, F. Fornara, Q. Fan, I. Searle, A. Giakountis, S. Farrona, L. Qissot, C. Turnbull, G. Coupland- 2007-FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*- Science 316: (5/18) 1030-1033

Leslie M- 2007-At long last, pathologists hear plants' cry for help- Science 318: (#5847, 10/5) 31-32

Here is review of the concept of the **apoplastic space** in plants.

Canny M.J- 1995-Apoplastic water and solute movements: New rules for an old space- Annual Review of Plant Physiology and Plant Molecular Biology 46: 215-236

BIO 107   2010
Lecture 24, Title: Eukaryotic diversity II: Chordates and Vertebrates
Text Readings: Campbell et al. (2008), pgs. 445-446, 513, 525-528, 654-655, 661-663, chapter 34, and Appendix E.

Topics to cover:

> **Definition of Animals**
> **Groups leading to us and distinctive traits**
> > **Deuterostomes**
> > **Chordates**
> > **Craniates**
> > **Vertebrates**
> > **Gnathostomes**
> > **Osteichythes**
> > **Lobe-Finned Fish**
> > **Tetrapods**
> > **Amniotes**
> > **Mammals**
> > > **Monotremes**
> > > **Marsupials**
> > > **Eutherians**
> > > **Primates**

Definition of Animals
Wish to show how we (primates) fit in to the animal kingdom.
> To do this we will define animals
> Then will note the groups of which we are a part and the distinguishing traits
It is difficult to make generalizations, each group's description will have exceptions...
> So many qualifications will be made, and expect things to be altered....
> > i.e. if there is an argument for reinterperting the lineage then new traits will be
> > > emphasized in the future....
> But the relationships we will cover seem fairly solidly worked out... so far.
Relationships and shared traits matter... Very useful when trying to study functions in us.
> By understanding where humans fit in the Animal Kingdom we gain perspecitive.
> > Also can identify model organisms for study, ones that model some aspect of us.
Who are animals? (Ruppert et al. 1994)
> Animals are metazoans. > 35 phyla, very diverse
> They are:
> > eukaryotes, with multicellular diploid stage (2N), heterotrophic
> > that have a blastula stage in their embryo development   Fig. 32.2, blastula
> > Have interupted meiosis in egg production (oogenesis).
> > Common evolutionary lineage (most important!)

Why exclude each of the following groups?

    protists: also heterotrophic; but no blastula, etc

    fungi: also heterotrophic, different life cycle

ultimately exclude because these are not same lineage....

Some issues I have with text (pgs. 654-655)

    Text says: Animals are ingestive?

        take items into a digestive chamber? But sponges do not do this?

        So not all animals are ingestive? thus this trait is not inclusive enough?

        Contrast ingestion and digestion. Later is also done by fungi...

    Text says: Animals lack cell walls?

        Cell walls are part of the extracellular matrix, and animals have ECM.

        Tunicates are animlas who have a "celluloselike" tunic

            Fig. 35, adult tunicate

        Animals have a complex extracellular matrix, like plants and fungi, protists

            this is necessary for multicellularity?

        Cell wall is a type of ECM, so this is a distinction of the type of ECM?

    Text says: Animals have muscles and nervous tissue?

        This is not likely to have been true of oldest common ancestor?

            not all animals have these tissues, i.e. sponges

        Protists also use cytoskeleton to move at cellular level

            actin and myosin are found in protists...

        Other eukaryotes have cells that carry action potentials

        Better to define kingdom with common traits

            common to hypothetical common ancestor, may be lost later

        In this case muscles and nervous tissues are found in "higher" animals,

            but are not a good common trait to look for in an ancestor?

        better not to focus on traits also found in other lineages.

                i.e. some plants are heterotrophic

    Text says: Animals have Hox genes and homeoboxes.

        There are homeotic genes found in animals.

        But have been reported in non-aminals:

        (Rosin etal., 2001; Tomero etal., 1996; Jorgensen etal. 1999)

        My guess is that not been looked for in many other eukaryotes?

        Can use genetic definition, but have to be specific.

            Emphasis on Hox genes good, on homoetic genes bad...?

            So animals do have a type of homeotic gene set called Hox genes..

Animal Groups leading to us and distinctive traits

    Will cover major traits and a few groups in Animal Kingdom of which we are a part.

    This will follow groups in text closely.

    Should know traits, groups, examples of species that are in each group,

        and consider how the new traits allow a species to be able to live in new habitats.

Deuterostomes
Fig. 32.11, animal phylogeny
skipping many larger groups here, and many other branches...
three tissue layers present, ectoderm, mesoderm, endoderm (i.e. triploblastic)
bilateral body symmetry (compared to radial like a jellyfish)
internal body cavities (coelom), relate to our body cavities (i.e. abdominal, pericardial...)
complete digestive tract, second mouthed, meaning the mouth forms second
    .... same as protoanal? meaning the anus forms first....
early indeterminate cleavage

Chordates
  Fig. 34.1, Chordates (Campbell and Reece, 2003)
    (see fig. 34.2, deuterostomes and chordata in text...)
This is a Phylum, our phylum: Phylum Chordata
There are over 35 phyla in the animal kingdom
  Fig. 34.3, chordate characteristics
Notochord: An internal secretion that forms an extracellular structure spanning the body.
    Importance of structural support. Like cartilage in stiffness.
Dorsal-hollow nerve cord: Dorsal to notochord. Early part of CNS.
Pharyngeal slits: Initially for food capture and gas exchange.
Postanal tail: Tail useful for movement. So tend to be motile...
  All these traits are seen in humans, but highly modified
    or so altered during our development that they are not always obvious.
    Notochord remains as cushioning around our vertebrae
    Our spinal cord is dorsal and hollow
    Our postanal tail is reduced but a few bones of it are still present
    Pharyngeal slits used for forming other structures in us, as we will see...
Examples
  Urochordata, tunicates
    Fig. 34.5, Urochordata, tunicates
    blue tunicate.jpg (Steene, 1998)
    blue tunicates.jpg (Steene, 1998)
Paedogenesis,   retention of characteristics from early stages in development
  Fig. 34.4, lancelet; Fig. 34.5c, tunicate larva
  compare to tunicate larva to lancelet, early stage compared to adult stage
  some argue for this concept, some against...

Craniates

This is a subphylum, Subphylum Craniata. As in having a cranium around the brain.

Fig. 34.2, (Campbell and Reece, 2003)

Fig. 34.9, Hagfish

    note hagfish cartilaginous cranium present, but no vertebrae

    Neural crest cells are present, leading to peripheral nerves in PNS

    cartilagenous notochord, and cartilagenous skull, no bones present

    A Craniate, but not a vertebrate

    Duplication of Hox genes associated with cranium...

        gene duplication allows new roles at different stages of development

Vertebrates

Fig. 34.1, Chordates (Campbell and Reece, 2003)

vertebral column, found in vertebrates, not in Craniates

Fig. 34.10, Lamprey

    A vertebrate, but has cartilage not mineralized bones in vertebrae initially

    So vertebrae means there are units for structural support, not necessarily boney

    Other genes duplicated with vertebrae formation...

Gnathostomes

Have jawed mouths

Fig. 34.2, Chordate phylogeny

    jaws, teeth and other mineralized items start to show up, not just cartilage

    duplication of Hox genes again, now up to four sets...

    Example:

        Fig. 34.15, black tip shark, stingray, ratfish

            mineralized teeth, but no bones

    Fig. 34.13, evolution of the jaw

    Note jaw evolution, a modification of pharyngeal slit structures

        around each slit would expect supporting cartilage,

        that cartilage and opening modified to take on a new role

Osteichythes

Fig. 34.2, Chordate phylogeny (Campbell and Reece, 2005)

    Some gnathostomes have mineralized skeletons (bony fish)

    Osteocytes are cells of connective tissue, present now to create bones...

    Example: ray-finned fish

        (Fig. 34.16, fish)

    "lung-derivatives" outpockets of digestive tract, form swim bladder

        this helps adjust buoyancy, useful for free swimming fish

Also have other items we have today from fish.

    Shubin 2009 - hernia and testes pg 66. jpg

        Our testis's ducts wrap around our pelvic bone due to how testes

            descended. Basis for this is from our fish ancestors

        This promotes hernia in males. So, fish do not get hernias!

    Shubin 2009 - Hiccups pg 67.jpg

        Hiccups based on nerves used by lungfish

        needed to move water past gills and not into lungs

        so stopped up glottis when moving water.

        Our hiccups are a circuit we use that is based on this system.

Lobe-Finned Fish

    Fig. 34.2, Chordate phylogeny (Campbell and Reece, 2005)

    Some bony fish got lobed-fins, with bone and muscle in them

        this allows better ability to push against much,

        so perhaps shallow water dwellers?

    Tips of fins may still be ray-like.

    Example, coelacanth

        Fig. 34.18, Coelocanth

        lobe-fins, note flesh in fins, muscles and bone in them

Tetrapods

    two sets of appendages modified as legs, terrestrial, from fleshy fins

        hense: tetrapods "four feet"

    from fish stuck in ponds or lakes? Needed to push through shallow waters?

    Consider frogs, have stage when need water

            gills at tadpole stage

            external fertilization

    Fig. 34.19, tetrapod structure

            Amphibians are tetrapods

            Note pectoral and pelvic girdles for muscle attachment points.

                need to have an anchor against which force is applied

    Figs. 34.21 and 22, amphibian diversity

            frogs, salamanders, etc.

    Not all tetrapods are terrestrial,

    How are terrestrial tetrapods adaptive to this new habitat?

        Tetrapod traits make little sense in water.

            Ok to have a set of four fleshy fins, but ray fins work just as well?

Amniotes

Fig. 34.2, Chordate phylogeny (Campbell and Reece, 2005)

anmiotic egg, a response to stress of terrestrial living?

Fig. 34.25, amniotic egg

review structures of anmiotic egg, recall fetal pig....

embryo

extra-embryonic structures

amnion, allantois, yolk sac, chorion

recall functions of each...

Each is a tissue, made of many cells, not a biological membrane

Examples:

Fig. 34.24, amniotes

note reptiles, and mammals

So can now do internal fertilization, no need for a pond to mate in

embryo does not need a pond to develop in.

These are adaptive to drier terrestrial habitats. Further from abundant waters.

Mammals

The Mammals are a class: Class Mammalia.

mammary glands, making milk

hair made of keratin

mostly endothermic

4 chambered heart (note birds have this to, so it evolved twice?)

Monotremes

Fig. 34.35, orders of mammals

monotremes, lay eggs, lack nipples

Marsupials

Placental mammals

have nipples for feeding of very small young as finish growth

Eutherians

Also placental mammals, birth of more developed young

also have nipples for feeding young

Examples:

Fig. 34.36, mammal phylogeny (Campbell and Reece, 2005)

note both Eutherians and Marsupials are placental mammals

eutherians use the placenta longer

Primates, an order related to rats and rabbits

Fig. 34.35, orders of mammals

Order Primates, opposable thumb for tree dwelling...

Fig. 34.39, apes

Review major groups and traists (see fig. 34.2).
> (Fig. 34.2, Campbell and Reece, 2005)
> Know the characteristics of these groups, examples of animals in them.
>> Consider what traits each has that is similar to our traits, and how these traits make the animals with them better fit for life in certain habitats.....
> Relate the groups covered here to the classical classification system (see appendix E)
>> Kingdom Animalia
>> Phylum Chordata
>> Subphylum Craniata
>> Class Mammalia
>> Order Primates

Objectives:

What characteristics distinguish each of the following groups? Be able to name specific examples of members of each group. Also consider how the traits found in a group influences the types of habitats in which they live or the types of lifestyles they display.

Animals
Deuterostomes
Chordata
Craniates
Vertebrates
Gnathostomes
Osteichthyans
Lobe-finned fish
Tetrapods
Amniotes
Mammals
Monotremes
Marsupials
Eutherians

Be able to describe the characteristics of the amniotic egg. How have the parts of the egg been modified amongst various amniotic species?

What are examples of traits that have appeared during the course of animal evolution that are thought to involve the duplication of genes?

Be able to describe a model for the evolution of the jaw, and what preexisting structures were modified to make it?

For review see self-quiz questions #2-6 of chapter 34.

Needed overheads and items:

Fig. 32.2, blastula
Fig. 34.5, adult tunicate
Fig. 32.11, animal phylogeny
Fig. 34.1, Chordates (Campbell and Reece, 2003)
Fig. 34.3, chordate characteristics
Fig. 34.5, Urochordata, tunicates
blue tunicate.jpg (Steene, 1998)
blue tunicates.jpg (Steene, 1998)
Fig. 34.5c, tunicate "tadpole",
Fig. 34.4, lancelet
Fig. 34.2, Chordate phylogeny (Campbell and Reece, 2005)
Fig. 34.9, Hagfish
Fig. 34.1, Chordates (Campbell and Reece, 2003)
Fig. 34.10, Lamprey
Fig. 34.2, Chordate phylogeny (Campbell and Reece, 2005)
Fig. 34.15, black tip shark, stingray, ratfish
fig. 34.13, evolution of the jaw
Fig. 34.2, Chordate phylogeny (Campbell and Reece, 2005)
Fig. 34.16, fish
Shubin 2009 - hernia and testes pg 66.jpg
Shubin 2009 - Hiccups pg 67.jpg
Fig. 34.2, Chordate phylogeny (Campbell and Reece, 2005)
Fig. 34.18, Coelocanth
Fig. 34.19, tetrapod structure
Fig. 34.21
Fig. 34.22, amphibian diversity
Fig. 34.2, Chordate phylogeny (Campbell and Reece, 2005)
Fig. 34.25 amniotic egg
Fig. 34.24, amniotes
Fig. 34.35 orders of mammals
Fig. 34.6, mammal phylogeny (Campbell and Reece, 2005)
Fig. 34.35, orders of mammals
Fig. 34.39, apes
Fig. 34.2, Chordate phylogeny (Campbell and Reece, 2005)

Handout:
animals.tif

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Vertebrates. Chapter 34. And pgs. 445-446, 513, 525-528, 654-655, 661-663, and Appendix E. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-Biology, 7th edition, Figures 34.2, 34.6. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2003-Biology, 6th edition, Figures 34.1, 34.2, Benjamin Cummings Press. San Francisco, CA.

Jorgensen J.E., M. Gronlund, N. Pallisgaard, K. Larsen, K.A. Marcker, J.E. Ostergaard- 1999-A new class of plant homeobox genes is expressed in specific regions of determinate symbiotic root nodules- Plant Molecular Biology 40: (#1) 65-77.

Rosin F. M., D.J. Hannapel, J.K. Hart- 2001- A potato homeobox gene (POTH1) involved in plant development- Plant Biology 60:

Ruppert E.E., R.D. Barnes- 1994-Sponges and Placozoans. Chapter 3, in Invertebrate Zoology. Saunders College Publishing, Fort Worth. Pages 68-94.

Shubin N.H- 2009-This old body- Scientific American 311; (#1, Jan.) 64-67

Steene R- 1998-Coral Seas. 272 pgs. Firefly Books Inc. Buffalo N.Y.

Tornero P., V. Conejero, P. Vera- 1996-Phloem-specific expression of a plant homeobox gene during secondary phases of vascular development- Plant-Journal 9: (#5) 639-648

Related issues:

How much do we share with other **primates**? Here are some studies that examine social and **cognitive abilities**, the origins of **bipedalism**, and other issues relating to primate evolution. Also, there have been recently new discoveries of human relatives, and possible **ancestors** we share with the apes.

Balter M- 2010-Candidate human ancestor from South Africa sparks praise and debate- Science 328: (#5975, 4/9) 154-155

Bennett M.R., J.W.K. Harris, B.G. Richmond, D.R. Braum, E. Mbua, P. Kiura, D. Olago, M. Kibunjia C. Omuombo, A.K. Behrensmeyer, D. Haddart, S. Gonzalez- 2009- Early hominin foot morphology based on 1.5 million-year-old footprints from Ileret, Kenya- Science 323: (#5918, 2/27) 1197-1201

Berger L.R., D.J. de Ruiter, S.E. Churchill, P. Schmid, K.J. Carlson, P.H.G.M. Dirks, J.M. Kibil- 2010-*Australopithecus sediba*: A new species of *Homo*-like australopith from South Africa- Science 328: (#5975, 4/9) 195-204

Gibbons A- 2010-Human ancestor caught in the midst of a makeover- Science 328: (#5977, 4/23) 413

Gibbons A- 2009-Break through of the year: *Ardipthecua ramidus*- Science 326: (#5960, 12/18) 1598-1599

Gibbons A- 2007-Spear-wielding chimps seen hunting bush babies- Science 315: (2/23) 1063

Harris R.A., J. Rogers, A. Milosavljevic- 2007-Human-specific changes of genome structure detected by genomic triangulation- Science 316: (4/13) 235-237

Herrmann E., J. Call, M.V. Hernández-Lioreda, B Hare, M. Tomasello- 2007-Humans have evolved specialized skills of social cognition: The cultural intelligence hypothesis- Science 317: (#5943, 9/7) 1360-1366

McGrew W.C- 2010-Chimpanzee technology- Science 328: (#5978, 4/30) 579-580

O'Higgins P., S. Elton- 2007-Walking on trees- Science 316: (6/1) 1292-1294

Lovejoy C.O- 2009-Reexamining human origins in light of *Ardipithecus ramidus*- Science 326: (#5949, 10/2) 74 (74e1-74e8)

Silk J.B- 2007-Social components of fitness in prmate groups- Science 317: (#5843, 9/7) 1347-1351

Stringer C., C. Gamble- 1993-Who were the Neanderthals- Chapter 1, pgs. 12-38, in In search of the Neanderthals. Solving the puzzle of human origins. Thames and Hudson Inc., N.Y.

Thorpe S.K.S., R.L. Holder, R.H. Crompton- 2007-Origin of human bipedalism as an adaptation for locomotion on flexible branches- Science 316: (6/1) 1328-1331

Van Schaik G- 2006-Why are some animals so smart?- Scientific American 294: (#4, Apr) 64-71

Wong K- 2009-The human pedigree- Scientific American 300: (#1, Jan) 60-63

Wood J.N., D.D. Glynn, B.C. Phillips, M.D. Hauser- 2007-The perception of rational, goal-directed action in nonhuman primates- Science 317: (#5843, 9/7) 1402- 1405

They have detected **neandertal genes** in some modern humans, which suggests someone in the past did some cross breeding (to find out if it was your ancestor, see the following... ;-).

Gibbons A- 2010-Close encounters of the prehistoric kind- Science 328: (#5979, 5/7) 680-684

Green R.E., J. Krause, A.W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, H. Li, W. Zhai, M.H-Y. Fritz, N.F. Hansen, E.Y. Durand, A-S. Malaspinas, J.D. Jensen, T. Marques-Bonet, C. Alkan, K. Prüfer, M. Meyer, H.A. Burbano, J.M. Good, R. Schultz, A. Aximu-Petri, A. Butthof, B. Höber, B. Höffner, M. Siegemund, A. Weihmann, C. Nusbaum, E.S. Lander, C. Russ, N. Novod, J. Affourtit, M. Egholm, C. Verna, P. Rudan, D. Brajkovic, Ž. Kucan, I. Gušic, V.B. Doronichev, L.V. Golovanova, C. Lalueza-Fox, M. de la Rasilla, J. Fortea, A. Rosas, R.W. Schmitz, P.L.F. Johnson, E.E. Eichler, D. Falush, E. Birney, J.C. Mullikin, M. Slatkin, R. Nielsen, J. Kelso, M. Lachmann, D. Reich, S. Pääbo- 2010-A draft sequence of the Neandertal genome- Science 328: (#5979. 5/7) 710-722

One of the recent findings has been of fossils of the so-called **"hobbits"** (*Homo florensis*). Here are some articles relating to this branch of the hominins. (I have not found any reports of elf fossils.... yet!

Culotta E- 2009-Did humans learn from hobbits?- Science 324: (#5926, 4/24) 447

Culotta E- 2008-When hobbits (slowly) walked the earth- Science 320: (#5875, 4/25) 433-435

Culotta E- 2007-The fellowship of the hobbit- Science 317: (#5839, 8/10) 740-742

Gibbons A- 2007-Hobbit's status as a new species gets a hand up- Science 316: (4/6) 34

Tocheri M.W., C.M. Orr, S.G. Larson, T. Sutikua, Jatmiko, E.W. Saptomo, R.A. Due, T. Djubiantono, M.J. Morwood, W.L. Jungars- 2007-The primitive wrist of *Homo floresiensis* and its implications for hominin evolution- Science 317: (#5845, 9/21) 1743-1745

Wong K- 2009-Rethinking the Hobbits of Indonesia- Scientific American 301: (#5, Nov.) 66-73

There articles describe studies of the evolution of the **mammalian inner ear** through modification of ancestral **gill slits**.

Ji Q., Z-X. Luo, X. Zhang, C-X. Yuan, L. Xu- 2009-Evolutionary development of the middle ear in mesozoic therian mammals- Science 326: (#5950, 10/9) 278-281

Martin T., I. Ruf- 2009-On the mammalian ear- Science 326: (#5950, 10/9) 243-244

Ancient **vertebrates**, **animal ancestors** and their traits:

Benton M.J- 2000-Vertebrate origins- Chapter 1, pgs. 1-14, in- Vertebrate Palaeontology. 2nd edition. Blackwell Science. Oxford, U.K.

Brusca G.J., R.C. Brusca, S.F. Gilbert- 1997-Characteristics of metazoan development- Chapter 1, pgs 3-19, in Embryology: Constructing the organism. S.F. Gilbert and A.M. Raunio editors. Sinauer Assoc., Sunderland, MA.

Callier V., J.A. Clack, P.E. Ahlberg- 2009-Contrasting developmental trajectories in the earliest known tetrapod forelimbs- Science 324: (#5925, 4/17) 364-367

Friedman M- 2009-Emerging onto a tangled bank- Science 324: (#5925, 4/17) 341-342

Shen B., L. Dong, S. Xiao, M. Kowalewski- 2008-The Avalon explosion: Evolution of Ediacara morphospace- Science 319: (#5859, 1/4) 81-84

Shu D-G., H-L. Luo, S.C. Morris, X-L. Zhang, S-X. Hu, L. Chen, J. Han, M. Zhu, Y. Li, L-Z. Chen- 1999-Lower Cambrian vertebrates from South China- Nature 402: (Nov. 4) 42-46

Travis J- 2007-A close look at urbisexuality- Science 316: (4/20) 390-391

Wong K- 2003-An ancestor to call our own- Scientific American 288: (#1) 54-63

They have found new fossils of **therapods**, that might be the ancestor of the bird lineage.

Choiniere J.N., X. Xu, J.M. Clark, C.A. Foster, Y. Guo, F. Han- 2010-A basal alvarezsauroid theropod from the early late Jurassic of Xinjiang, China- Science 327: (#5965, 1/29) 571-574

Theses articles describe some of the conditions in the **ancient oceans** at the time when the **evolution of the animal kingdom**.

Narbonne G.M- 2010-Ocean chemistry and early animals- Science 328: (#5974, 4/2) 53-54

Li C., G.D. Love, T.W. Lyons, D.A. Fike, A.L. Sessions, X. Chu- 2010-A stratified redox model for the Ediacaran ocean- Science 328: (#5974, 4/2) 80-83

For more on **Hox genes** and other **gene duplications** in animals see:

Lemons D., W. McGinnis- 2006-Genomic evolution of Hox gene clusters- Science 313: (9/29) 1918-1922

Murphy W.J., D.M. Larkin, A. Everts-van der Wind, G. Bourque, G. Tesler, L. Auvil, J.E. Beever, B.P. Chowdhary, F. Balibert, L. Gotzke, C. Hilte, S.N. Meyers, D. Milan, E.A. Ostrander, G. Pape, H.G. Parker, T. Raudsepp, M.B. Rogatcheva, L.B. Schook, L.C. Skow, M. Welge, J.E. Womack, S.J. O'Brien, P.A. Pevzner, H.A. Lewin- 2005-Dynamics of mammalian chromosome evolution inferred from multispecies comparative maps- Science 309: (7/22) 613-617

Popesco M.C., E.J. MacLaren, J. Hopkins, L. Dumas, M. Cox, L. Meltescen, L. McGavran, G.J. Wyckoff, J.M. Sikela- 2006-Human lineage-specific amplification, selection, and neuronal expression of DUF1220 domains- Science 313: (9/1) 1304-1307

BIO 107   2010
Lecture 25, Title: Movement II
Text Readings: Campbell et al. (2008) pgs. 1112-1117, fig. 40.5 (connective and muscle tissues), and the chapter on Biomechanics in the BIO 107 Lab Manual.

Topics to cover:
     Use of analysis of skeletons
     Review law of lever
         mechanical advantage
         speed advantage
         compressive forces
     Joints and muscle connections
     Movement examples
         Running and jumping
         Digging
         Flying
     Summary

Use of analysis of skeletons
     Want to be able to look at skeletons and tell something about the life of the animal.
     pg. 555, Sabretooth cat skull (Hildebrand and Goslow, 2001)
         Sabretooth cats are extinct, so can not directly determine strength of bite
     Fig. 4.9, skeletons of ancestral birds (Hildebrand and Goslow, 2001)
     When looking at fossils of birds. Which could fly, and which could not?
     Was a dinosaur fast or slow as a runner? How did it move?
     How to design prosthetic limbs?
         Musser 2008 - prosthetic arm.jpg (Musser, 2008)
     To determine these sorts of things we need to know principles of biomechanics related to joint/muscle systems in animals. Can do this by examining living animals and then applying these principles to fossil skeletons. Similar issues occur in plants, and other multicellular organisms.

Review law of lever

     Will start with physical principles
     Torque in = Torque out
     $F_i S_i = F_o S_o$ forces and distances of input and output
         muscle insertion point versus lever output force application point
         if muscle pulls and is inserted it must be anchored somewhere...
     force generated by muscles, distances determined by attachment points and joints
     Fig. 22.6, examples of lever systems (Hildebrand and Goslow, 2001)
         Good examples of input and output of forces
         Notice that the foot can be several lever types, depends on use...
     A few other principles can be determined from the law of the lever:

mechanical advantage

High force output. Mechanical advantage = $F_o/F_i = S_i/S_o$
> This is just a rearrangement of the law of the lever

A good example is seen in the jaw.

Fig. 8.25, skull of wolf (Hildebrand and Goslow, 2001)
> Dark areas are where muscles were located.
>> One opens jaw, two shown to close jaw
> Compare input to output distances, especially for back teeth
>> think about using teeth to crack a nut versus eating bread...

Note that can always have several muscles working across the same joint...

speed advantage

High speed output. Speed advantage = $S_o/S_i = F_i/F_o$
> Another way the law of the lever can be rearranged

Fig. 24.8, insertion point and rotation (Hildebrand and Goslow, 2001)
> Hind limb of a lizard, insertion at A would give less swing.
> For high speed output want shorter muscle insertion relative to joint

Fig. 24.11, summation of swing of joints in limb (Hildebrand and Goslow, 2001)
> Final output speed need not be due to just one joint, can be due to several

Notice that MA and SA are invertedly related, can not maximize both at one joint

compressive forces

Bone and joint must withstand the forces applied to them, both input and resistance forces

If the bone shatters, that is not adaptive...

How to withstand compressive forces?

Fig. 23.4, Elephant foreleg (Hildebrand and Goslow, 2001)
> thick bones
> bone contact points are broader than shaft of bone, spreads out force

The law of the lever and considering mechanical advantage, speed advantage, and
> compression forces allows us to analyze joints.

Joints and muscle connections

Consider actual muscles and joints.

Extensors and flexors:

Fig. 50.32, muscles acting at joints
> origins vs. insertion points of muscles
> contrast extensor vs. flexor,
>> muscles only pull, so need antagonistic pairs of muscles
> Note insertion and origin points of each muscle in above.

Tendons and Ligaments:

Fig. 21.5, fox forelimb (Hildebrand and Goslow, 2001)

note tendons for connections of muscles, ligaments for bones

    Muscles attach to bones via tendons

    Ligaments attach bones to bones, and group the tendons.

Tendons and ligaments are both types of connective tissue.

Fig. 21.6, horse ligaments (Hildebrand and Goslow, 2001)

    this figure shows use of ligaments to hold neck against gravity

        the ligaments can be stretchy, so horse can use muscles to move its neck

Multiple muscles can work together to create a common output at a joint.

Fig. 22.1, summation of forces at one joint (Hildebrand and Goslow, 2001)

    Most joints have more than one extensor muscle, as this joint here

    So have to sum the forces of each to get resulting force

Movement examples

Now we consider patterns of bones and lever systems associated with various styles of movement.

Running and jumping

Running:

Fig. 24.3, examples of running (Hildebrand and Goslow, 2001)

    will consider running four-legged animals

Fig. 24.5, Hind limbs, bear vs. dog vs. deer (Hildebrand and Goslow, 2001)

    consider effect of lengthening of limb on SA.

    Draw in hypothetical extensor and flexor muscles.

    Where would you put them to optimize SA?

        Consider the mass of the muscle, takes force to move it

            so positioning muscle close to body is easier and faster?

    Given the length of these bones who is most likely to be fastest? Sum over joints.

Fig. 24.6, Deer vs. Beaver ribs and clavicle (Hildebrand and Goslow, 2001)

    notice how the freeing of the shoulder blade in deer allows limb to swing in one plane of movement for running.

    Consider shoulder blade of dog, cat, vs. our shoulder blade.

Fig. 22.9, storing energy in ligaments of spine (Hildebrand and Goslow, 2001)

    Also allows spine to flex, and adds to extension

Fig. 24.7, cheetah running (Hildebrand and Goslow, 2001)

    Notice gains from: rotation of limbs, and extending of spine

Fig. 24.9, comparison of faster-runners vs. slower-runners (Hildebrand and Goslow, 2001)

    Compare SAs via output distances to input distances relative to joints

        Cheetah is faster than lion, 4.45 > 3.36

        Agouti is faster than muskrat, 5.48 > 4.30

        Kangaroo is faster than wombat, 4.64 > 3.50

Summary of Adaptations for running:
>   high SA joints, flexible spine with stretchable ligaments,
>
>   shoulder and hip rotation in one plane
>
>   thin limbs with muscle mass near body center

Jumping:

Fig. 24.2, examples of jumping mammals (Hildebrand and Goslow, 2001)

Fig. 29.2, ligaments of foot of horse (Hildebrand and Goslow, 2001)

>   acts to store energy in stride, via stretchable ligaments
>
>   notice width of bones where meet, dealing with compression forces here

Fig. 4.19, early frog skeletons (Benton, 2000)

>   several joints move at once, fusion of vertebrae for better support

Summary of Adaptations for jumping:

>   springy ligmanents
>
>   fusion of basal vertebrae if vertical jumper
>
>   thicker bones/joints to deal with compression forces

Digging

Another type of movement that has evolved several times in various lineages.

Fig. 25.2, examples of diggers (Hildebrand and Goslow, 2001)

>   This trait has evolved separately several times.

Will consider mainly the mole.

Fig. 25.3, humeral-rotational digging of moles (Hildebrand and Goslow, 2001)

>   note humerus (upper arm) rotated to move ulna (lower arm)
>
>   Try showing how this works with human arm...
>
>   Notice, shoulder does not flex very much.

Fig. 25.9, mole skeleton (Hildebrand and Goslow, 2001)

>   note large phalanges (finger bones) for shovel-blade like hand for digging
>
>   large muscles inserted on spine and shoulder
>
>   insert on humerus and on ulna

Fig. 25.5, adaptations for digging (Hildebrand and Goslow, 2001)

>   Thick bones to handle compression forces, short and stubby for high MA
>
>   long elbow gives a long insertion arm
>
>   shovel-like hands can deal with stresses of digging

Fig. 25.6, adaptations for digging (Hildebrand and Goslow, 2001)

>   additional examples, note long elbows
>
>   shoulders are somewhat fixed, do not rotate much

Summary Adaptations for digging:

>   High MA joints, thicker bones, rigid shoulder, broad fingers, large muscles.

Flying
Another style of movement that has evolved several times
        We are concerned here just with vertebrates, but insects can also fly...
Fig. 28.18, examples of flight (Hildebrand and Goslow, 2001)
Fig. 10.17, Bird muscles (Hildebrand and Goslow, 2001)
        Note Pectoralis muscle, lowers wing for downstroke
                Supracoracoid muscle, elevates wing
                both anchor on sternum
Fig. 10.18, bird muscles, soaring bird (Hildebrand and Goslow, 2001)
        Supracoracoid is small and inserts on upper side of humerus
        pectoralis is large for powerful stroke, inserts on lower side of humerus
Fig. 28.20, bat vs. pheasant skeletons (Hildebrand and Goslow, 2001)
        Fusion of sections of the spine
        Shoulder braced by clavicles or coracoids, need to have wings lift body
        large fused sternum with keels for muscle origins, and stiff body
        With high SA, have low MA, so need large muscles and strong anchor points
Fig. 28.19, Soaring vs. hovering bird wing skeletons (Hildebrand and Goslow, 2001)
        Variation in manner of flight.
        Note how skeletons differ across style
        So from skeleton can tell if it would fly, and perhaps style of flight used?
Fig. 28.2, Pterosaur (Hildebrand and Goslow, 2001)
        Application of these principles to a fossil
Fig. 8.20, Pterosaurs (Benton, 2000)
        note sizes range for various species of pterosaurs
Fig. 28.3, wings of bird, Pterosaur, and bat (Hildebrand and Goslow, 2001)
        reduction of parts, fusion leads to elimination of finger and wrist bones
        long and thin, bird lengthens the wing further by using feathers
Summary of Adaptations for flight:
        muscle mass proximal to body, reduction of distal mass in limb
        fusion/loss of bones, high SA and low MA so need big flight muscles

Summary

Do not memorize names of muscles or bones.
Know: examples of high MA, SA systems; be able to apply the law of the lever.
        adaptations for running, digging, flight.
        how to attach muscles around a joint for extensor or flexor action.
With these principles we can analyze a fossil skeleton. Can then determine:
        *its mode of movement.
        *something about its lifestyle, and much more....

Objectives:

Contrast the connections and uses of tendons versus that of ligaments.

Be able to use the law of the lever in calculations to rank lever systems in terms of their relative mechanical or speed advantages. If given a joint, be able to propose a possible flexor or extensor muscle for it by indicating the possible anchorage and insertion points for each muscle relative to that joint. Consider how the muscle and its attachment points could be changed to improve either the mechanical advantage, or the speed advantage, of that joint.

Be able to describe examples of biological joints that are relatively high in mechanical advantage, or high in relative speed advantage. How are these arrangements adaptive for the animal that has it?

Be able to describe specific examples of animals that dig, run, or fly. Be able to describe adaptations that are evident in the skeletons and muscles of animals that use each one of these types of movement. If shown several skeletons be able to compare them in terms of specific adaptations present in them that would make them adaptive for digging, or running, or flight.

How are bones and joints altered to deal with compressive forces?

Needed overheads and items:

pg. 555, Sabretooth cat skull (Hildebrand and Goslow, 2001)
Fig. 4.9, skeletons of ancestral birds (Hildebrand and Goslow, 2001)
Musser 2008 - prosthetic arm.jpg
Fig. 22.6, examples of lever systems (Hildebrand and Goslow, 2001)
Fig. 8.25, skull of wolf (Hildebrand and Goslow, 2001)
Fig. 24.8, insertion point and rotation (Hildebrand and Goslow, 2001)
Fig. 24.11, summation of swing of joints in limb (Hildebrand and Goslow, 2001)
Fig. 23.4, Elephant foreleg (Hildebrand and Goslow, 2001)
Fig. 50.32, muscles acting at joints
Fig. 21.5, fox forelimb (Hildebrand and Goslow, 2001)
Fig. 21.6, horse ligaments (Hildebrand and Goslow, 2001)
Fig. 22.1, summation of forces at one joint (Hildebrand and Goslow, 2001)
Fig. 24.3, examples of running (Hildebrand and Goslow, 2001)
Fig. 24.5, Hind limbs, bear vs. dog vs. deer (Hildebrand and Goslow, 2001)
Fig. 24.6, Deer vs. Beaver ribs and clavicle (Hildebrand and Goslow, 2001)
Fig. 22.9, storing energy in ligaments of spine (Hildebrand and Goslow, 2001)
Fig. 24.7, cheetah running (Hildebrand and Goslow, 2001)
Fig. 24.9, comparison of faster-runners vs. slower-runners (Hildebrand and Goslow, 2001)
Fig. 24.2, examples of jumping mammals (Hildebrand and Goslow, 2001)
Fig. 29.2, ligaments of foot of horse (Hildebrand and Goslow, 2001)
Fig. 4.19, early frog skeletons (Benton, 2000)
Fig. 25.2, examples of diggers (Hildebrand and Goslow, 2001)
Fig. 25.3, humeral-rotational digging of moles (Hildebrand and Goslow, 2001)
Fig. 25.9, mole skeleton (Hildebrand and Goslow, 2001)
Fig. 25.5, adaptations for digging (Hildebrand and Goslow, 2001)
Fig. 25.6, adaptations for digging (Hildebrand and Goslow, 2001)
Fig. 28.18, examples of flight (Hildebrand and Goslow, 2001)
Fig. 10.17, Bird muscles (Hildebrand and Goslow, 2001)
Fig. 10.18, bird muscles, soaring bird (Hildebrand and Goslow, 2001)
Fig. 28.20, bat vs. pheasant skeletons (Hildebrand and Goslow, 2001)
Fig. 28.19, Soaring vs. hovering bird wing skeletons (Hildebrand and Goslow, 2001)
Fig. 28.2, Pterosaur (Hildebrand and Goslow, 2001)
Fig. 8.20, Pterosaurs (Benton, 2000)
Fig. 28.3, wings of bird, Pterosaur, and bat (Hildebrand and Goslow, 2001)

Handout:
Handout Lecture 25 - Movement II (file with selected figures for students....)

References:

Benton M.J- 2000-<u>Vertebrate Palaeontology</u>. 2nd edition. 389 pgs. Blackwell Science. Oxford, U.K. Figures 4.19, 8.20.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Sensory and Motor Mechanisms. Chapter 50. Pages 1112-1117. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Hildebrand M., G. Goslow- 2001-<u>Analysis of Vertebrate Structure</u>. 5th edition. John Wiley & Sons, Inc. N.Y., N.Y. 635 pgs. Figures 4.9, 8.25, 10.17, 10.18, 21.5, 21.6, 22.1, 22.6, 22.9, 23.4, 24.2, 24.3, 24.5, 24.6, 24.7, 24.8, 24.9, 24.11, 25.2, 25.3, 25.5, 25.6, 25.9, 28.2, 28.3, 28.18, 28.19, 28.20, 29.2, pg. 555.

Musser G- 2008-Making them whole. Artificial limbs and a prosthetic arm create a path to better bionics- Scientific American 298: (#1, Jan) 52-53

Related issues:

Here are some examples of uses of **biomechanics** in the study of living **animals**.

Hanna J.B., D. Schmitt, T.M. Griffin- 2008-The energetic cost of climbing in primates- Science 320: (#5878, 5/16) 898

Ijspeert A.J., A. Crespi, D. Ryczko, J-M. Cabelguen- 2007-From swimming to walking with a salamander robot driven by a spinal cord model- Science 315: (3/9) 1416-1420

Liao J.C., D.N. Beal, G.V. Lauder, M.S. Triantafyllou- 2003-Fish exploiting vortices decrease muscle activity- Science 302: (Nov. 28) 1566-1569

Maladen R.D., Y. Ding, C. Li, D.I. Goldman- 2009-Undulatory swimming in sand: Subsurface locomotion of the sandfish lizard- Science 325: (#5938, 7/17) 314-318

Pennisi E- 2007-Crab's downfall reveals a hole in biomechanics study- Science 315: (1/19) 325

Pennisi E- 2007-Muscle fibers shift into high gear- Science 315: (1/26) 456-457

Pennisi E- 2007-Robot suggests how the first land animals got walking- Science 315: (3/9) 1352-1353

Wigglesworth V.B- 1984-Muscles and movement- Chapter 9, pgs. 145-153, in <u>Insect Physiology</u>. Chapman and Hall Publishers, London.

Young J., S.M. Walker, R.J. Bomphrey, G.K. Taylor, A.L.R. Thomas- 2009-Details of insect wing design and deformation enhance aerodynamic function and flight efficiency- Science 325: (#5947, 9/18) 1549-1552

For more on the study of **animal fossils** and their **biomechanics** see:

Braddy S.J- 2003-Trackways - Arthropod locomotion- Chap. 4.1.6, pgs. 389-393, in <u>Palaeobiology II</u>. D.E.G. Briggs and P.R. Crowther editors. Blackwell Pub. Oxford, U.K.

Clark J- 2009-Becoming *T. rex*- Science 326: (#5951, 10/16) 373-374

Fox S- 2009-Leapin' lizards. Biomechanics suggest how a giraffe-sized pterosaur took flight- Scientific American 300: (#5, May) 25-26

Lockley M.G- 2003-Trackways - Dinosaur locomotion- Chap. 4.1.11, pgs. 408-412, in <u>Palaeobiology II</u>. D.E.G. Briggs and P.R. Crowther editors. Blackwell Pub. Oxford, U.K.

Lovejoy C.O., B. Latimer, G. Suwa, B. Asfaw, T.D. White- 2009-Combining prehension and propulsion: The foot of *Ardipithecus ramidus*- Science 326: (#5949, 10/2) 72 (72e1-72e8)

Lovejoy C.O., G. Suwa, L. Spurlock, B. Asfaw, T.D. White- 2009-The pelvis and femur of *Ardipithecus ramidus*: The emergence of upright walking- Scinece 326: (#5949, 10/2) 71 (71e1-71e6)

Sereno P.C., L. Tan, S.L. Brusatte, H.J. Kriegstein, X. Zhao, K. Cloward- 2009-Tyrannosaurid skeletal design first evolved at small body size- Science 326: (#5951, 10/16) 418-422

They have recently found fossils of **dinosaur feathers** and can estimate the colors they may have had.

Li Q., K-Q. Gao, J. Vinther, M.D. Shawkey, J.A. Clarke, L. D'Alba, Q. Meng, D.E.G. Briggs, R.O. Prum- 2010-Plumage color patterns of an extinct dinosaur- Science 327: (#5971, 3/12) 1369-1372

And this article proposes how **flight** may have evolved in vertebrates.

Nudds R.L., G.J. Dyke- 2010-Narrow primary feather rachises in *Confuciusornis* and *Archaeopteryx* suggest poor flight ability- Science 328: (#5980, 5/14) 887-889

Here are some articles dealing with biomechanical issues in **plants**:

Briand C.H., A.D. Daniel, K.A. Wilson, H.E. Woods- 1998-Allometry of axis length, diameter, and taper in the Devil's Walking stick (*Aralia spinosia*; Araliaceae)- American Journal of Botany 85: (#9) 1201-1206

Farquhar G., Y. Zhao- 2006-Fracture mechanics and its relvance to botanical structures- American Journal of Botany 93: (#10) 1449-1454

Koehler L., F.W. Telewski- 2006-Biomechanics and transgenic wood- American Journal of Botany 93: (#10) 1433-1438

Moulia B., C. Coutand, C. Lenne- 2006-Posture control and skeletal mechanical acclimation in terrestrial plants: Implications for mechanical modeling of plant architecture- American Journal of Botany 93: (#10) 1477-1489

Niklas K.J- 1992-Plant Biomechanics: An engineering approach to plant form and function. 607 pages. University of Chicago Press. Chicago, IL.

Niklas K.J., H.C. Spatz, J. Vincent- 2006-Plant biomechanics: An overview and prospectus- American Journal of Botany 93: (#10) 1369-1378

Read J., A. Stokes - 2006-Plant biomechanics in an ecological context- American Journal of Botany 93: (#10) 1546-1565

Schopfer P- 2006-Biomechanics of plant growth- American Journal of Botany 93: (#10) 1415-1425

Skotheim J.M., L. Mahadevan- 2005-Physical limits and design principles for plant and fungal movements- Science 308: (5/27) 1308-1310

Spatz H-C., L. Köhler, T. Speck- 1998-Biomechanics and functional anatomy of hollow-stemmed spenopsids. I. *Equisetum giganteum* (Equisetaceae)- American Journal of Botany 85: (#3) 305-314

Speck T., N.P. Rowe- 2003-Plant growth forms and biomechanics- Chap. 4.1.3, pgs. 379-384, in Palaeobiology II. D.E.G. Briggs and P.R. Crowther editors. Blackwell Pub. Oxford, U.K.

BIO 107   2010
Lecture 26, Title: Study of molecular diversity
Text Readings: Campbell et al., (2008), pgs. 97, 405 (fig. 20.9).

Topics to cover:

> **Importance of molecular isolation**
> **Properties of organic molecules used for separation**
> **Cell homogenization, issues and options**
> **Three Techniques for Separation of molecules**
>> **Centrifugation**
>> **Gel Electrophoresis**
>> **Chromatography**
> **Summary**

Importance of molecular isolation

>Isolated molecules allows study of:
>>its location and function, its structure, how it is made/degraded,
>Can also design probes/tests to detect it. (recall enzyme lab and spectrophotometer?)
>>Fig. 5.15, growth cone cytoskeletal fluorescent probes (Sanes et al., 2006)
>>For instance antibodies can be produced to bind specific items,
>>>and can be modified to fluoresce so can detect location in cell.
>Can study regulation of function of proteins,
>>do *in vitro* studies of functions, (*in vitro* = outside of life...)
>>example: *in vitro* DNA replication systems require many isolated components
> As with all the compartments of the cell, if we are going to try to define what it is we need to
know what makes it made up. Work done by Kiesel (1930), on the slime mold *Reticularia* sp.,
found that the dehydrated protoplasm consisted of the following parts: (Kiesel.jpg)

substance	percent of dry weight
protein	28
nucleic acids	4
other nitrogen containing compounds	12
fats	18
lecithin	5
cholesterol	1
carbohydrates	23
other	9

> Composition does not tell function, but it is a beginning.

1

If can separate the molecules by type, then can study their functions

For proteins, functions include:

Ability to carry out catalysis (i.e. enzymes)

Ability to bind other things (binding sites that are not active sites)

Properties of organic molecules used for separation

A variety of properties are used to separate molecules:

size: Glucose versus starch, or ATP versus DNA

shape: Long and linear (DNA), or round (many cytosolic proteins)

mass per volume: density differs with elemental composition

charge: Under what pH does its functional groups take on a charge?

hydrophobicity: Solubility in water relative to oil.

Cytosolic proteins versus membrane ones. May relate to charge and pH?

enzymatic activity, binding sites: Makes a molecule sticky as binds certain items.

Hexokinase and glucose binding site...

Criteria of purity: If a sample of molecules stay together when subjected to techniques

that attempt to separate by different physical properties then it <u>might</u> be pure...

i.e. may be all the same type of molecule in the sample.

Use at least TWO different molecular properties to test for purity.

Once separate them, how do you detect them? Need for stains, ability to absorb light, or

other systems?

Otherwise are limited to items with distinct colors, smells, or tectures?

Cell homogenization, issues and options

One human cell may easily have over 120000 distinct types of molecules.

there may be only a few of the molecule type you want per cell

can be just one hormone receptor per cell...

So may need to start with large amounts of tissue, many cells.

First isolation of some hormones may use tens of Kg of starting material.

Cell homogenization: Have to break cell open and pull out items.

Freeze: Use dry ice, or liquid nitrogen, or regular freezer.

Sonication: Use of sound waves to vibrate and rupture cells.

Osmotic shock: Cause cell to swell by osmosis and burst.

Grinding and slicing: From morter and pestle, to blenders...

Issues during homogenization:
Digestive enzymes in endosomal organelles are often released,
will cut up items you wish to isolate.
Can try to protect your item by giving alternat substrates
To protect from proteases use BSA (Bovine Serum Albumin)
Isolation medium:
Do you wish to denature all enzymes, or perserve their activities?
What conditions?
temperature, pH, ionic concentrations, cofactors,
redox potential, inhibitors of hydrolytic enzymes... etc...?

Three Techniques for Separation of molecules
In crude homogenate have lots of congealed masses.
Need to separate these to get what you wish out of it.
For instance, in lab will use a petroleum ether/acetone phase separation to do this..

Centrifugation
Separates by differences in density (mass per volume).
Fig. 6.5, cell fractionation
initial crude separation, good for bulk organelle separation
Fig. 8.9, velocity vs. equilibrium sedimentation
(Alberts etal., 2002) centrifugation.jpg
Sucrose-density gradient, can determine density from this
Each layer of sucrose has a different concentration and so a different density
Item with density equal to a layer will not be pulled further down,
items lighter will rise, items denser will sink
Useful for separating organelles...

Gel Electrophoresis
Fig. 20.9, gel electrophoresis
separates by charge to mass ratio, so two factors here... mass is related to size
Changes in pH can alter charge...
gel with pores, various types of gels used
Starch, agarose, acrylamide
differ in pore sizes made, so useful for different ranges of molecular sizes
electrical field, applied across gel
net charge on proteins
but what if protein has no net charge? Can alter pH.
what if protein has net positive charge?
Fig. 8.14, (Alberts etal., 2002) SDS_page1.jpg

SDS = sodium dodecyl sulfate
      interacts with protein
      gives similar charge per unit mass
      with charge covered, separation of protein is mainly by mass (i.e. size)
    Use of beta-mercaptoethanol
      can cleave -S-S- bonds in protein, disrupts quaternary structure
      Consider effects of this on insulin?
Fig. 8.13, (Alberts etal., 2002) SDS + mercaptoethanol SDS_mercaptoethanol.jpg
Can use SDS-PAGE system to check the purity of the sample.
    Fig. 8.15, (Alberts etal., 2002) SDS_page2.jpg
shows stages of purification by each lane from a different stage in separation
Recall general criteria of purity
    see one band/peak when separated on the basis of two properties
    so would take the last lane here and use some other technique on it.

Chromatography
Various types, can do separation by size, solubility, or binding ability...
column chromatography, an imobile matrix held in a column...
    Fig. 8.10, (Alberts etal., 2002) chromatography1.jpg
      running sample solution through column, this is the mobile phase
      Can wash sample through column
      column has small beads in it
        high Surface area, this column material is the immobile phase
        so interacts well or not with molecules in sample
      separation is based on the interaction of molecules and beads
        If interaction is strong/often then sample moves very little
        If interaction is weak/rare then sample moves faster
          as will be in mobile phase more often
Three specific types of column chromatography are commonly used:
    Fig. 8.11, (Alberts etal., 2002) chromatography2.jpg filtration, ionic, affinity
      Filtration chromatographic system; separates by size
        beads have pores of known size
        traps small molecules as they enter the pores more often
        lets larger ones to go through as they do not go in pores
        Thus larger molecules move through faster..
        can purchase beads with many sizes
      Ion exchange chromatography; separates by charge
        beads have net charge
        interacts with charge on molecules
        binds opposite charged molecules
        lets ones with similar charge pass
        once caught molecules with a charge
          can pass through salt and dislodge them

Affinity chromatography; separates by binding sites
analog of substrate fixed to beads
proteins with ability to bind
once bound can pass through unbound
substrate to knock proteins off
These show examples of the sort of separations that can be achieved
Fig. 8.10, (Alberts etal., 2002) chromatography1.jpg
Fig. 8.12, (Alberts etal., 2002) chromatography3.jpg
describe the results of filtration, ionic, affinity seen here
Once have separate samples still need to detect desired item
so need to do enzyme assay, or check protein content etc...
Thin layer chromatography
phase separation, separates by hydrophobicity
note oil/water partitioning, relate to phase partitioning will do in lab...
(draw example on board)
Fig. 8.22, (Freifelder, 1982) TLC.jpg
interaction of item with each phase, immobile phase vs. mobile phase
immobile phase, bound to plastic or glass, may be paper, hydrophilic
mobile phase, washes over immobile phase, may be organic solvents
sample put on a spot on immobile phase before start partitioning.

Summary
Depending on properties of molecule trying to isolate, have various methods to purify it.
Often have to use several approaches all together.
Criteria of purity.

Still have issues of:
How to detect what you have separated?
How to confirm that the isolated item is intact and functioning normally?

For instance, it can often take several years to purify a desired hormone's receptor, and after that can begin to study its regulation activity... more job security...

Objectives:

Be able to list several properties of molecules that can be used to separate them from each other. What is a separation technique that could be used to separate items according to each molecular property?

What would be differences in the approach taken when trying to isolate a hormone versus a receptor for that hormone? Be able to describe differences in the molecular abundance and properties of a hormone versus its receptor, and how this might influence the approaches and techniques of isolation that might have to be used.

Identify and describe ways to get around some of the problems involved in isolating and purifying a molecule intact out of a tissue of many cells. Why is one method of purification rarely sufficient to achieve a completely pure sample of one type of molecule?

What information about a molecule can be obtained by each of the following techniques? Centrifugation, chromatography (consider the various column and thin-layer types), and electrophoresis. Be able to describe broadly how each of these techniques works, including the molecular property that each uses to achieve molecular separation.

Needed overheads and items:

Fig. 5.15, growth cone (Sanes et al., 2006)
Kiesel (1930) table of cell chemical composition, Kiesel.jpg
Fig. 6.5, cell fractionation
Fig. 8.9, velocity vs. equilibrium sedimentation (Alberts etal., 2002) centrifugation.jpg
Fig. 20.9, gel electrophoresis
Fig. 8.14, (Alberts etal., 2002) SDS_page1.jpg
Fig. 8.13, (Alberts etal., 2002) SDS + mercaptoethanol SDS_mercaptoethanol.jpg
Fig. 8.15, (Alberts etal., 2002) SDS_page2.jpg
Fig. 8.10, (Alberts etal., 2002) chromatography1.jpg running sample through column
Fig. 8.11, (Alberts etal., 2002) chromatography2.jpg filtration, ionic, affinity
Fig. 8.10, (Alberts etal., 2002) chromatography1.jpg
Fig. 8.12, (Alberts etal., 2002) chromatography3.jpg results of filt., ionic, affinity
Fig. 8.22, (Freifelder, 1982) TLC.jpg

Handout: (Lect 26 - handouts.stm)
Fig. 8.9, velocity vs. equilibrium sedimentation (Alberts etal., 2002) centrifugation.jpg
Fig. 8.11, (Alberts etal., 2002) chromatography2.jpg filtration, ionic, affinity

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002-<u>Molecular Biology of the Cell</u>. 4th edition. Figures 8.9, 8.10, 8.11, 8.12, 8.13, 8.14, 8.15, 8.20. Garland Science Press. N.Y., N.Y.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008- Pages 97 and 405. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Freifelder D- 1982-<u>Physical Biochemistry: Applications to Biochemistry and Molecular Biology</u>. 2nd edition. Fig. 8.22. W.H. Freeman and Co., San Francisco, CA.

Kiesel A. 1930. Chemie des Protoplasmas. Protoplasmatologia Monograph 4, Berlin.

Sanes D.H., T.A. Reh, W.A. Harris- 2006-Axon growth and guidance- Fig. 5.15, in <u>Development of the Nervous System</u>, 2nd edition. Elsevier Academic Press, N.Y., N.Y.

Related issues:

Some other methods and issues involved with the **isolation of molecules** and study of their properties:

> Pestov D., R.K. Murawski, G.O. Ariunbold, X. Wang, M. Zhi, A.V. Sokolov, V.A. Santenkov, Y.V. Rostovtsev, A. Dogarium, Y. Huang, M.D. Scully - 2007- Optimizing the laser-pulse configuration for coherent Raman spectroscopy- Science 316: (4/13) 265-268
>
> Pregibon D.C., M. Toner, P.S. Doyle- 2007-Multifunctional encoded particles for high-throughput biomolecule analysis- Science 315: (3/9) 1393-1396
>
> Thor G- 2009-Preparing the proteome for mass spectrometry- Science 323: (#5917, 2/20) 1080-1082
>
> Xue Q., E.S. Yeung- 1995-Differences in the chemical reactivity of individual molecules of an enzyme- Nature 373: (Feb 23) 681-683

Here is an example of a study that uses **electrophoresis**:

> Canut H., S. Baudracco, M. Cabane, A.M. Boudet, G. Marigo- 1991-Preparation of sealed tonoplast and plasma-membrane vesicles form *Catharanthus roseus* (L.) G. Don. cells by free-flow electrophoresis- Planta 184: 448-456

This is a simple, easy, **electrophoresis** system you can set up at home:

> Carlson S- 1998-Sorting molecules with electrophoresis- Scientific American 279: (#6) 110-111

These two papers use **centrifugation**:

> Scherer G.F.E., G. Fischer- 1985-Separation of tonoplast and plasma membrane H^+-ATPase from zucchini hypocotyls by consecutive sucrose and glycerol gradient centrifugation- Protoplasma 129: 109-119
>
> Terry M.E., B.A. Bonner- 1980-An examination of centrifugation as a method of extracting an extracellular solution from peas, and its use for the study of indole acetic acid- Plant Physiology 66: 321-325

This paper makes use of **chromatography**:

> Long A.R., J.L. Hall, L.E. Williams- 1997-Chromatographic resolution, purification and characterization of H^+-PPase and H^+-ATPase from *Ricinus* cotyledons- Journal of Plant Physiology 151: 16-24

BIO 107   2010
Day 12, Lecture 27, Title: Action potentials.
Text Readings: Campbell et al. (2008), pgs. 1047-1056.

Topics to cover:
       Role of animal nervous system
       Parts of a neuron, and molecular players
       Events of an Action Potential
       Enhancing the speed of Action Potential Transmission
       Role of Action Potentials in Non-Animals

Role of animal nervous system
       With a multicellular organism need coordination and integration
       One approach to this is via hormones, but that takes time
       A faster system can be useful
       Across cells there is a way to generate an electromagnetic wave down length of cell
              Some of these changes are action potentials
       Rapid signals allows faster signal integration and response...

Parts of a neuron, and molecular players
             Fig. 48.4, vertebrate neuron
       dendrites: site of reception of signals from other cells
       cell body: synthesis of neurotransmitters, and also receives signals from other cells
       axon: carries Action Potential and at end sends signals to other cells
              Fig. 48.5, neuron shapes
       variation in neurons: note branching, the number of connections can be huge
The molecular players needed to set up and generate an action potential
             Fig. 48.6, ion set up before action potential, and transport systems
       Note locations of important transport systems in different regions of the plasma membrane
       ion concentration gradients, and ion pump (Na^+/K^+-ATPase)
              can use Na^+/X symports or antiports as well to alter the membrane potential
       "leak" channels set resting membrane potential, mainly Potassium ion channels
              membrane potential drives other ion movements through other "leak" channels
       note ion pump is slow (100s/sec), channels are quick (millions/sec)
       membrane potential
              Fig. 48.8, measuring membrane potential of neuron
              remember that molecules move in response to ENERGY gradient!
                    ions respond to local electrical field (membrane potential)
                        and to local concentration gradient of that ion
                        These comprise the ion's electrochemical gradient
       ion channels act to allow specific types of ions to move across membrane
              activity of ion channels can be altered by ligand binding, or voltage sensing...
              anything that alters the ion channel shape can be used to alter its activity

What happens to membrane potential if ions move?

Example on board: vesicle with ions, electrodes in and out to monitor
movement of Cl^-, Na^+, or K^+ across membrane
show what each does to membrane potential measured
Some terms are used to describe changes in membrane potential
Fig. 48.9, graded potentials and action potential
depolarization: towards 0 mV, either from positive or negative side of it
threshold potential: point at which voltage-sensitive Na^+ channels are activated
hyperpolarization: away from 0 mV, either on positive or negative side
Action potential: involves a specific sequence of changes in membrane potential
done in a repeated fashion and in set amout of time for a cell type
With neuron at rest, before action potential,
go through sodium, potassium, chloride movements
then put in context of energy gradients across membrane
Concept of voltage sensitive ion channels (sodium ion and potassium ion channels)
contrast ion channels sensitive to binding (ligand-gated ion channels)
note locations of these on neuron, axon hillock and along axon to axon terminus

Events of an Action Potential
Action potential characteristics
An all-or-none pattern of changes in membrane potential
Has non-varying properties: (exceptions, can vary across development or species)
Amplitude change is set
Duration is set
[Draw on board: Membrane Potential (E_m) vs. position down length of cell's axon
draw out Action Potential stages as move down cell]
Fig. 48.10, events in action potential
threshold potential
near axon hillock the action potential can start once past threshold here
depolarization activates sodium ion channels, ultimately induces a hyperpolarization
voltage-sensitive Na^+ channels are activated once past threshold
This alters the polarity of the membrane in this area.
This change in electrical field influences other areas AT THE SPEED OF LIGHT
the further away the weaker is its influence
hyperpolarization alters the activity of potassium ion channels
sodium ion channels close when membrane potential is positive
Positive membrane potential is sensed by voltage-sensitive K^+ channels, these open
note how change in electrochemical K^+ gradient alters K^+ movement
compare to resting potential state
movement of K^+ will return membrane potential towards normal and beyond

refractory period, also called undershoot

        Membrane potential is hyperpolarized, and need time to reset the ion channels

        Note this prevents backwards movement of action potential

                Relate to sequence down an axon,

                        one spot is finishing action potential

                        another is in the middle of action potential

                        another spot futher down is just passing threshold potential...

Note how ions move laterally, across the cell membrane.

        Fig. 48.11, ion movements during Action Potential

        Ions do NOT flow down the length of the cell, this figure is a bit misleading?

This gives a means to send a wave of electronegativity rapidly down the length of a cell.

        Faster than cytoplasmic streaming, circulatory system, or diffusion.

Enhancing the speed of Action Potential Transmission

Saltatory conduction

        Fig. 48.12, Schwann cells and sheaths

        an unfortunate term, "Saltatory" means discontinuous, does not refer to salts....

        So influence of action potential jumps from place to place, discontinuous

influence of capacitance

        capacitance = # charges per voltage change; inversely proportional to width

                analogous to buffering of pH, a measure of resistance to change

        low capacitance means

                moving a few charges gives larger voltage change

                so a voltage difference at one point has influence further down cell

        high capacitance means

                have to move more charges to get same voltage change

                so smaller steps, as a voltage difference has less influence

Change capacitance of membrane by myelin sheath, makes capacitance lower...

        called saltatory conduction (jump from place to place)

These sheaths lower the local capacitance,

        Fig. 48.13, nodes and sheaths

        only place that need to induce are nodes of Ranvier

                and at these nodes the action potentials are generated

                This limits area of membrane that has to be activated

                and since areas are at a distance makes transmission faster

Note error fig 48.13: ions do not move along axon length to transmit Action Potential

        Neurons are up to meters long

        If ions moved down length would take hours to days!

        Ions move laterally, in/out of cell in response to local electrochemical gradients

                this is 10 nm distance, movement is very rapid across this distance

        Electrical fields transmit at speed of light down length of axon

                has to influence next section of membrane past threshold

                this is much faster than ion movement can be

Role of Action Potentials in Non-Animals

The use of electromagnetic waves sent down cell length is found in many eukaryotes
So animals did not come up with action potentials, nor are they found only in neurons...

Action potentials occur in plants. They move through the phloem and the symplast.
Can go from leaves to roots, or roots to leaves.
based on different ion movements: Cl^-, Ca^{+2} as well as Na^+ and K^+
slower than animal A.P.s: in potatoes can be 0.07-7 cm/sec
Venus' flytrap uses action potential to signal for trap closure.
(Students should see why hormonal signaling would not work as well.)

Algae have action potentials. *Chara* was one of the first species in which
action potentials were worked out in detail.

Also occur in paramecia! Why, I am not sure...

The use of a sequence of membrane ion channel activity to alter the membrane potential is
something seen in many eukaryotic species, not just animals.

So we have a means to get information concerning reception of a signal(s) at one end of a neuron to the other end of the neuron.

In next lectures will cover how events at synapse act to induce action potential at the axon hillock, and how neurons communicate with other cells across the synapse.

Objectives:

Be able to describe the sequence of events involved in the propagation of an action potential, and note which of these help define action potentials as an "all-or-none" phenomenon.

What are the different parts of a vertebrate neuron called? What type(s) of ion channels are present in the plasma membrane of a typical neuron, and where in the cell are they located?

Before an action potential what is a typical magnitude of the neuron's membrane potential? What are the relative concentrations of K^+ and Na^+ inside of, and outside of, the cell before an action potential? Be able to describe how these ions got put into in this distribution and how this relates to the resting potential the cell produces. What active transport system is needed to make these conditions be present so that an action potential can happen in a nerve cell? What passive transport systems are needed to establish the resting potential across the membrane? What passive transport systems are used during the action potential?

What is hyperpolarization, and how does it differ from depolarization? If Na^+ enters a typical nerve cell which is at rest, will the membrane potential hyperpolarize or depolarize? What if the Na^+ left the cell? What would happen if Cl^- entered or left the cell?

Why is it difficult to start a new action potential at a section of a cell's membrane that is experiencing a refractory period? Be able to describe how the firing of one action potential induces a new action potential further down the axon, but not back towards the cell body.

Be able to describe the effect of a myelin sheath on the rate of propagation of an action potential. Be able to describe saltatory conduction, and how the action potential is propagated from node to node down an axon.

What are some non-neuron cells that have action potentials in animals? What are some non-animal species that have action potentials? What features of these other action potentials would be similar to those seen in animal neurons? What would a non-animal species be doing with their action potentials?

For review see self-quiz questions #1, 2, 3, 7 and 8 at the end of this chapter.

Needed overheads and items:

        Fig. 48.4, vertebrate neuron
        Fig. 48.5, neuron shapes
        Fig. 48.6, ion set up before action potential
        Fig. 48.8, measuring membrane potential of neuron
        Fig. 48.9, graded potentials and action potential
        Fig. 48.10, events in action potential
        Fig. 48.11, ion movements during A.P. (note direction of ion movement?)
        Fig. 48.12, Schwann cells and sheaths
        Fig. 48.13, nodes and sheaths

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Neurons, Synapses, and Signaling. Chapter 48. Pages 1047-1056. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Related issues:

For more on the **ion channels** activities involved in action potentials, and for examples of how their characteristics are measured see the following:

Alle H., A. Roth, J.R.P. Geiger- 2009-Energy-efficient action potentials in hippocampal mossy fibers- Science 325: (#5946, 9/11) 1405-1408

Hille B- 1984-Ionic channels of excitable membranes. 425 pgs. Sinauer Associates Inc. Sunderland, MA.

Shepherd G.M- 1988-The action potential- Chapter 6, pgs. 101-121, in Neurobiology, 2nd edition. Oxford University Press, N.Y.

Tao X., J.L. Avalos, J. Chen, R. Mackinnon- 2009-Crystal structure of the eukaryotic strong inward-rectifier K^+ channel Kir2.2 at 3.1 Å resolution- Science 326: (#5960, 12/18) 1668-1674

Changes in **membrane potentials** have been observed in **artificial systems**, such as veiscles, implying that membrane potential changes may have occurred in the simpliest of cells.

Ishima Y., A.T. Przybylski, S.W. Fox- 1981-Electrical membrane phenomena in spherules from proteinoid and lecithin- BioSystems 13: 243-251

These studies note that **action potentials** can extend up into **dendrites** as well as down axons, and so could influence activities of receptors in the dendrites.

Hu H., M. Martina, P. Jonas- 2010-Dendritic mechanism underlying rapid synaptic activation of fast-spiking hippocampal interneurons- Science 327: (#5961, 1/1) 52-58

Lorincz A., Z. Nusser- 2010-Molecular identity of dendritic voltage-gated sodium channels- Science 328: (#5980, 5/14) 906-909

Action potentials are useful for signaling in a multicellular organism. Therefore they are not jsut found in animals. For more on action potentials in **plants** and their uses there see:

Fromm J., H. Fei- 1998-Electrical signaling and gas exchange in maize plants of drying soil- Plant Science 132: 203-213

Fromm J., M. Hajirezaei, I. Wilke- 1995-The biochemical response of electrical signaling in the reproductive system of *Hibiscus* plants- Plant Physiology 109: 375-384

Johannes E., E. Ermolayeva, D. Sanders- 1997-Red light-induced membrane potential transients in the moss *Physcomitrella patens*: ion channel interaction in phytochrome signalling- Journal of Experimental Botany 48: (#S) 599-608

Trebacz K., A. Sievers- 1998-Action potentials evoked by light in traps of *Dionaea muscipula* Ellis- Plant and Cell Physiology 39: (#4) 369-372

Trebacz K- 1992-Measurements of intra- and extracellular pH in the liverwort *Conocephalum conicum* during action potentials- Physiologia Plantarum 84: 448-452

Volkov A.G., D.J. Collins, J. Mwesigwa- 2000-Plant electrophysiology: Pentachlorophenol induces fast action potentials in soybean- Plant Science 153: 185-190

For action potentials found in **algae** see the following studies:

Andjus P.R., O. Popović, N. Sarvazyan, M. Zivić, D. Vućelić-1997-Effects of ion transport inhibitors on D_2O induced action potential in Characeae- Plant Science 128: 121-129

Homann U., G. Thiel- 1994-Cl^- and K^+ channel currents during the action potential in *Chara*. Simultaneous recording of membrane voltage and patch currents- Journal of Membrane Biology 141: 297-309

Shimmen T- 1997-Studies on mechanoperception in Characean cells: Pharmacological analysis- Plant and Cell Physiology   38: (#2) 139-148

Staves M.P., R. Wayne- 1993-The touch-induced action potential in *Chara*: Inquiry into the ionic basis and the mechanoreceptor- Australian Journal of Plant Physiology 20: 471-488

Thiel G., U. Homann, C. Plieth- 1997-Ion channel activity during the action potential in *Chara*: New insights with new techniques- Journal of Experimental Botany 48: 609-622

Getting neurons to grow and regenerate is a major concern. Here is work on studies of axin and dendrite growth and on **axon regeneration**.

Atwal J.K., J. Pinkston-Gosse, J. Syken, S. Stawicki, Y. Wu, C. Shatz, M. Tessier-Lavigne- 2008-PirB is a functional receptor for myelin inhibitors of axonal regeneration- Science 322: (#5903, 11/7) 967-970

Hammarlund M., P. Nix, L. Hauth, E.M. Jorgensen, M. Bastiani- 2009-Axon regeneration requires a conserved MAP kinase pathway- Science 323: (#5915, 2/6) 802-806

Park K.K., K. Liu, X. Hu, P.D. Smith, C. Wang, B. Cai, B. Xu, L. Connolly, I. Kramvis, M. Sahin, Z. He- 2008-Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway- Science 322: (#5903, 11/7) 963-966

Shelly M., B.K. Lim, L. Cancedda, S.C. Heilshorn, H. Gao, M-m. Poo- 2010-Local and long-range reciprocal regulation of cAMP and cGMP in axon/dendrite formation- Science 327: (#5965, 1/29) 547-552

Sponges lack a complex nervous system, yet these animals also have been found to have action potentials.

Leys S.P., G.O. Mackie, R.W. Meech- 1999-Impulse conduction in a sponge- Journal of Experimental Biology 202: (#9, May) 1139-1150

BIO 107   2010
Day 12, Lecture 28, Title: Synapses

Text Readings: Campbell et al. (2008), pgs. 120-121, 1056-1061, 1078-1080.

Topics to cover:

> **Overview**
> **Electrical Synapses**
> **Chemical Synapses**
> **Inhibitory and Excitatory Synapses**
> **Neurotransmitter Types**
> **Modifications of Synapses**
> **If Time: Star-Nosed Mole**

Overview
> So far have action potentials going down one cell, a neuron.
> What induces the firing of an action potential?
> What will firing an action potential cause this cell to do?
> Will now cover events where two cells meet, and how information is integrated.
> A synapse:
>> Two cells, often chemical signals passed between mainly in one direction,
>> must be signals, receptors, and cell adhesion molecules, etc...

Electrical Synapses
> See electrical signals passed from cell to cell without chemical component.
> This depends on cytosolic connections...
> Low resistance allows for transmission of action potential cell to cell
> Plasmodesmata in plants, allow action potentials to pass cell to cell, via symplast
>> Fig 6.31
>> plasmodesmata.jpeg (Salisbury and Ross, 1992; fig. 7.8)
> Connections between fungal cells, pores between cells,
>> also allow action potential transmission
> Cardiac muscle cells
>> Fig. 6.32   have gap junctions that create groups of cell electrically joined
>> See this in heart
>> heart_muscles.jpeg (Marieb and Mallatt, 1997; fig. 17.4)
> This allows very fast speed of action potential transmission, but no integration/summation

Chemical Synapses
Recall, neuromuscular junction, it is a chemical synapse
fig. 50.29, skeletal muscle fiber
the post-synaptic cell here is a muscle cell, not a neuron
Fig. 48.15, chemical synapse

some parts of a synapse:
presynaptic cell, postsynaptic cell, synaptic vesicles (holding neurotransmitters),
receptors, ion channels, cell adhesion molecules,
neurotransmitter recovery systems and many many other proteins....
Transmission across takes milliseconds to seconds
depends on types of transmitters and receptors
run through sequence of events
action potential arrival, Ca^{+2} channel activation as it is voltage sensitive
Ca^{+2} concentration spike in axon terminal cytosol
neurotransmitter vesicle fusion, concentration of neurotransmitter in cleft,
neurotransmitter reception by post-synaptic cell, change in ion channel activity,
change in membrane potential in post-synaptic cell....
How vesicles are produced, recall endosomal system
Fig. 13.64,(Alberts etal, 2002) synaptic_vesicle_loading.jpg
vesicles often made in cell body, brought down axon
loaded at axon terminus, note recycling of vesicles
need to conserve membrane surface area....
Geometric factors and reuse of synapse
synaptic cleft, a space with small volume, so small amount of transmitter has large
effect on local concentration there
can concentrate receptors in one location for the neurotransmitter
Uptake systems, to recover transmitter so can use it again
Degrading systems, to remove transmitter
Or allow the neurotransmitter to diffuse away, this is very slow...

Inhibitory and Excitatory Synapses
multiple synapses can all influence one post-synaptic cell, and in various ways
Fig. 48.13a, (Campbell and Reece, 2002) multiple synaptic inputs
summation of signals is therefore possible at that one cell
Fig. 48.16, summation of post synaptic potentials
various types of synapses, implies variation in neurotransmitters and receptors
some are excitatory, some inhibitory
Also many types of neurotransmitters
Table 48.1, neurotransmitter types
some are modified amino acids, some are peptides, etc...

Modification of synapses over time

      Fig. 21.104, Alberts etal. (2002), synapses.jpg

      "neurons that fire together, wire together"

      temporal coordination plays a role in which synapses work together to

            strengthen or weaken the summation

      activity-dependent synapse elimination (also elimination of unused neurons...)

            those synapses that do not fire with other synapses tend to be eliminated

            those synapses that fire together tend to all persist together

Long-term potentiation

      changes in function with repeated use, seen in hippocampus of human brain

      Occurs at synapses that are used

            when post-synaptic cell is depolarized on repeated and rapid occasions

            with firing at pre-synaptic cell

      long term changes

            Fig. 49.20, vertebrate long term potentiation

            post-synaptic cell places more receptors at the stimulated synapse, so more

                sensitive to glutamate

                sends signals to pre-synaptic cell, NO (nitrous oxide)

                Alters activity of signal transduction systems

            pre-synaptic cell will release more vesicles and more glutamate

      non-NMDA receptors, are glutamate-gated ion channels

      Fig. 11.41, (Alberts etal., 2002), longtermpotentiation.jpg

            double-gated, receptor binds two items

                binding of glutamate is neurotransmitter reception

                membrane must be depolarized

                then NMDA receptor changes shape (so it is voltage sensitive)

                    this releases Mg^{+2} from inhibitory site

                    allows Ca^{+2} to pass, goes on to alter local area

                This leads to deployment of more AMPA receptors to this area

      Changes outside of synapses (Coggan et al., 2005)

            Have thousands of receptors per μm^2 at synapse,

                but have tens of receptors per μm^2 at non-synaptic areas.

            Also not all neurotransmitter vesicles are released at synapses.

            Not all reception is done at the synapses either.

            So non-synaptic signaling is possible...

Growing neurons and new synapses

      Growth cone concept

            spines, pseudopods, from growing cell use receptors for growth directions

            Fig. 48.33, direction of neruon growth (Campbell and Reece, 2005)

                use of signals on surface of cells

                part of signal-transduction, but signals are on cell surfaces

                proteins such as cell adhesion molecules (CAMs) involved

                    neuronal growth factors (NGF) also involved

            This implies receptors for these signals, we would like to know them

How to study this? Ability to grow cells in culture is critical
     can watch how they form new connections
     quicktime movie, 21.5, MBOTC, CD
     This gets to concept of neuronal stem cells, and using them to follow
          determination and differentiation of neuron cells
          (Yuang et al. 2002)
To observe growth of cell in culture or in situ use various techniques
     SEM (Harris, 1994)
     Confocal microscopy (Mills et al. 1994)
     Fluorescent microscopy (Jones et al. 1994a + b)
Example: (Davis, 2006) Davis 2006_Immune Synapse.jpg
     Examined synapses. Have circular structure.
     Neuronal synapses have receptors in center, with adhesion molecules
          around them. Creates small volume for NT release.
          So can get cell-to-cell specific signaling.
     Immune system cells have a similar system.
          Receptors in center, surrounded by adhesion molecules such as
          CDs
     Neuronal one is more stable, immune one transient, but use similar
          structural features? So synapses are using parts found in many cell
          types and in non-animals as well...

If time: Star-Nosed Mole
     Space race for use of brain.
     Catania (2002) *Condylura cristata*, Star-nosed mole
     Star nosed mole Cortical Map.jpg
          An organ for touch, note size of cortex for connections/processing
          competition during growth for space dedication in cortex
          if excise appendix 11, others take up space with connections
             A nice example of modification of neurons... implies new synapses
     Star nosed mole sense organ.jpg
          Shows nose
          Elements of touch on surface, Elmer's organs
          Each has touch receptors and neurons in it.

So synapses grow, change, connect, process, integrate. Are modifiable, not static.

Objectives:

What are the important structural and functional differences between chemical and electrical synapses. What structure(s) must be present between eukaryotic cells to allow for an electrical synapse to exist? What sort of structure(s) must be present, and where, for a chemical synapse to exist? Synapses are structures though which cell-to-cell communication occurs, what are some examples of cells in humans that carry out such communication across synapses?

Chemical synapses involve elements of signal reception and transduction systems. Be sure that you see this connection clearly and can identify the elements. In terms of just a single neuron's synapse, what is the signal, how is it transduced, and what might be the response of that cell?

What must happen in the presynaptic cell for it to transmit a signal to another cell? What then happens in the postsynaptic cell? The sequence of events, what structures are involved, the function of each, and how this can lead to the firing of an action potential should be clear to you (a close study of figure 48.15 might be useful here). Sending a signal across a synapse is one thing. How is the synapse reset so that the next signal can be sent?

Do all synapses result in the stimulation of an action potential in the postsynaptic cell? Do all synapses contribute equal effects on the postsynaptic cell? Can you describe how summation can work to produce complexity and information integration?

Be able to describe several ways in which the firing of a post-synaptic neuron could be modified over the long term.

Be able to propose several reasonable mechanisms for how a neuron could have its growth and formation of new synapses influenced by cells around it.

For review see self-quiz questions #4, 5, 6 at the end of chapter 48.

Needed overheads and items:

Fig. 6.31, plasmodesmata
plasmodesmata.jpeg   (Salisbury and Ross, 1992; fig. 7.8)
Fig. 6.32, gap junctions
heart_muscles.jpeg (Marieb and Mallatt, 1997; fig. 17.4)
Fig. 50.29, skeletal muscle fiber
Fig. 48.15, chemical synapse
Fig. 13.64, (Alberts etal, 2002) synaptic_vesicle_loading.jpg
Fig. 48.13a, (Campbell and Reece, 2002) multiple synaptic inputs
Fig. 48.16, summmantion of post synaptic potentials
Table 48.1, neurotransmitter types
Fig. 21.104, Alberts etal. (2002), synapses.jpg
Fig. 49.20, Vertebrate long term potentiation
Fig. 11.41, (Alberts etal., 2002), longtermpotentiation.jpg
Fig. 48.33, (Campbell and Reece, 2005) direction of neruon growth
quicktime movie, 21.5, MBOTC, CD
Davis 2006_Immune Synapse.jpg
Star nosed mole Cortical Map.jpg
Star nosed mole sense organ.jpg

Handout:
Fig. 13.64, (Alberts etal, 2002) synaptic_vesicle_loading.jpg

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Related issues:

Here are papers that describe of some of the events on the **presynaptic** side that are essential for **synapses function**.

Ferguson S.M., G. Brasnjo, M. Hayashi, M. Wölfel, C. Collesi, S. Giovedi, A. Raimond, L-W. Gong, P. Ariel, S. Paradise, E. O'Toole, R. Flavell, O. Cremona, G. Miesenböck, T.A. Ryan, P. DeCamilli- 2007-A selective activity-dependent requirement for dynamin 1 in synaptic vesicle endocytosis- Science 316: (4/27) 570-574

Martens S., M.M. Kozlov, H.T. McMahon- 2007-How synaptotagmin promotes membrane fusion- Science 316: (5/25) 1205-1208

Robinson P.J- 2007-How to fill a synapse- Science 316: (4/27) 551-553

How **memories** are formed is of interest. Though forgetting is just as important as learning, as long as it is selective. Changes at the synapse have been found to be associated with **loss of memory**.

Lee S-H., J-H. Choi, N. Lee, H-R. Lee, J-I. Kim, N-K. Yu, S-L. Choi, S-H. Lee, H. Kim, B-K. Kaang- 2008-Synaptic protein degradation underlies destabilization of retrieved fear memory- Science 319: (#5867, 2/29) 1253-1256

Shen H., N. Sabalianskas, A. Shorpa, A.A. Fenton, A. Stelzer, C. Aoki, S.S. Smith- 2010-A critical role for α4βδ GABA$_A$ receptors in shaping learning deficits of puberty in mice- Science 327: (#5972, 3/19) 1515-1518

Silva A.J., Y. Zhou, T. Rogerson, J. Shobe, J. Balaji- 2009- Molecular and cellular approaches to memory allocation in neural circuits- Science 326: (#5951, 10/16) 391-395

How are synapses removed? Some note a role for the **immune system** in helping to remove old unused synapses, and others study how the proteins in existing synapses are degraded.

Ding M., D. Chao, G. Wang, K. Shen- 2007-Spatial regulation of an E3 ubiquitin ligase directs selective synapse elimination- Science 317; (#5840, 8/17) 947-951

Miller D.M- 2007-Synapses here and not everywhere- Science 317: (#5840, 8/17) 907-908

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How synapses are made, **synaptogenesis**, is also of great interest.

Colón-Ramos D.A., M.A. Margeta, K. Shen- 2007-Glia promote local synaptogenesis through UNC-6 (Netrin) signaling in *C. elegans*- Science 318: (#5847, 10/5) 103-106

Matsuda K., E. Miura, T. Miyazaki, W. Kakegawa, K. Emi, S. Narumi, Y. Fukarawa, A. Ito-Ishida, T. Kondo, R. Shigemoto, M. Watanabe, M. Yuzaki- 2010-Cbln1 is a ligand for an orphan glutamate receptor δ2, a bidirectional synapse organizer- Science 328: (#5976, 4/16) 363-368

Patel M.R., K. Shen- 2009-RSY-1 is a local inhibitor of presynaptic assembly in *C. elegans*- Science 323: (#5920, 3/13) 1500-1503

Here is a study of what it takes to **maintain a synapse**. The process is highly regulated.

Williams A.H., G. Valdez, V. Moresi, Y. Qi, J. McAnally, J.L. Elliott, R. Bassel-Duby, J.R. Sanes, E.N. Olson- 2009-MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice- Science 326: (#5959, 12/11) 1549-1554

Errors in synapses have been associated with certain **illnesses**, such as autism and other syndromes.

Beeson D., O. Higuchi, J. Palace, J. Cossins, H. Spearman, S. Maxwell, J. Newsom-Davis, G. Burke, P. Fawcett, M. Motomura, J.S. Müller, H. Lochmüller, C. Slater, A. Vincent, Y. Yamanashi- 2006-Dok-7 mutations underlie a neuromuscular junction synaptopathy- Science 313: (9/29) 1975-1978

Garber K- 2007-Autism's cause may reside in abnormalities at the synapse- Science 317: (#5835, 7/13) 190-191

Snyder S.H- 2006-Adam finds an exciting mate- Science 313: (9/22) 1744-1745

Techniques now exist to monitor the use of individual **syanptic vesicles** in the presynaptic terminus. This has led to support of the "kiss-and-run" model of synaptic vesicle use.

Zhang Q., Y. Li, R.W. Tsien- 2009-The dynamic control of kiss-and-run and vesicular reuse probed with single nanoparticles- Science 323: (#5920, 3/13) 1448-1453

This study reports finding a rapid type of **EPSP** in some neurons of rats as they search an area.

Epsztein J., A.K. Lee, E. Chorev, M. Brecht- 2010-Impact of spikelet on hippocampal CA1 pyramidal cell activity during spatial exploration- Science 327: (#5964, 1/22) 474-477

How synapses can change over time is an active area of study. Here are some papers dealing with aspects of **synaptic plasticity**, including treatments and conditions that induce such changes.

Berghuis P., A.M. Rajnicek, Y.M. Morozov, R.A. Ross, J. Mulder, G.M. Urbán, K. Monory, G. Marsicano, M. Matteoli, A. Canty, A.J. Irving, I. Katona, Y. Yanagawa, P. Rakic, B. Lutz, K. Mackie, T. Harkany- 2007-Hardwiring the brain: Endocannabinoids shape neuronal connectivity- Science 316: (5/25) 1212-1216

Brecht M., D. Schmitz- 2008-Rules of plasticity- Science 319: (#5859, 1/4) 39-40

Clem R.L., T. Celikel, A.L. Barth- 2008-On going *in vivo* experience triggers synaptic metaplasticity in the neocortex- Science 318: (#5859, 1/4) 101-104

Dickman D.K., G.W. Davis- 2009-The schizophrenia susceptiblity gene *dysbindin* controls synaptic homeostasis- Science 326: (#5956, 11/20) 1127-1130

Farrant M., S.G. Cull-Candy- 2010-AMPA receptors - another twist?- Science 327: (#5972, 3/19) 1463-1464

Mameli M., B. Balland, R. Luján, C. Lüscher- 2007-Rapid synthesis and synaptic insertion of GluR2 for mGluR-LTD in the ventral tegmental area- Science 317: (#5837, 7/27) 530-533

Sanes D.H., T.A. Reh, W.A. Harris- 2006-Refinement of synaptic connections- Chap 9, pgs 247-287, in Development of the Nervous System, 2nd edition. Elsevier Academic Press, N.Y., N.Y.

Schwenk J., N. Harmel, G. Zolles, W. Bildl, A. Kulik, B. Heimrick, O. Chisaka, P. Jonas, U. Schulte, B. Fakier, N. Klöcker- 2009-Functional proteomics identify cornichon proteins as auxiliary subunits of AMPA receptors- Science 323: (#5919, 3/6) 1313-1319

Tiagaret C., D. Choquet- 2009-More AMPAR garnish- Science 323: (#5919, 3/6) 1295-1296

von Engelhardt J., V. Mack, R. Sprengel, N. Kavenstock, K.W. Li, Y. Stern-Bach, A.B. Smit, P.H. Seeburg, H. Monyer- 2010-CKAMP44: A brain-specific protein attenuating short-term synaptic plasticity in the dentate gyrus- Science 327: (#5972, 3/19) 1518-1522

Wang D.O., S.M. Kim, Y. Zhao, H. Hwang, S.K. Miura, W.S. Sossin, K.C. Martin- 2009-Synapse- and stimulus-specific local translation during long-term neuronal plasticity- Science 324: (#5934, 6/19) 1536-1540

Here are studies of how **calcium ions** are detected during the induction of **synaptic vesicle release**.

Groffen A.J., S. Martens, R.D. Arazola, L.N. Cornelisse, N. Lozovaya, A.P.H. deJong, N.A. Goriounova, R.L.P. Habets, Y. Takai, J.G. Borst, N. Brose, H.T. McMahon, M. Verhage- 2010-Doc2b is a high-affinity Ca^{2+} sensor for spontaneous neurotransmitter release- Science 327: (#5973, 3/26) 1614-1618

Lee H-K., Y. Yang, Z. Su, C. Hyeon, T-S. Lee, H-W. Lee, D-H. Kweon, Y-K. Shin, T-Y. Yoon- 2010-Dynamic Ca^{2+}-dependent stimulation of vesicle fusion by membrane-anchored synaptotagmin 1- Science 328: (#5979, 5/7) 760-763

Astrocytes are one type of cells that have been reported to have influences on local synaptic functions. **Glial cells** also have influences on synapses.

Bacaj T., M. Tevlin, Y. Lu, S. Shaham- 2008-Glia are essential for sensory organ function in *C. elegans*- Science 322: (#5902, 10/31) 744-747

Kirchhoff F- 2010-Questionable calcium- Science 327: (#5970, 3/5) 1212-1213

Rouach N., A. Koulakoff, V. Abudara, K. Willecke, C. Glaume- 2008-Astroglial metabolic networks sustain hippocampal synaptic transmission- Science 322: (#5907, 12/5) 1551-1555

Here is a study of the **bioenergetic costs** of **EPSP** and **IPSP** uses in summation. Such costs can be subjected to selection, and so are of interest in the study the evolution of this system.

Magistretti P.J- 2009-Low-cost travel in neurons- Science 325: (#5946, 9/11) 1349-1351

BIO 107   2010
Day 12, Lecture 29, Title: Nervous system.
Text Readings: Campbell et al. (2008), pgs. 1048, 1064-1078, 1080-1084.

Topics to cover:
> **Information Flow**
> **Knee-Jerk Reaction**
> **Neural Circuits**
> **Studies with *Aplysia***
> **Varieties of Nervous Systems**
> **Vertebrate Nervous System**
> > **Peripheral Nervous System**
> > **Central Nervous System**
> **Male versus Female Brains**
> **Plugging into the Brain**
> **Summary**

Information Flow
> sensory system, integration of information, response given
> Fig. 48.3, sensory, integration, response in neurons
> Note, this is signal - transduction - response over several cells through the organism
> Sensory cell: Not always a part of the nervous system, though it may be...
> > sensory neuron: Gets a signal from a sensory cell across a synapse.
> > interneuron: Neurons talking to neurons, the main neuron of the CNS.
> > motor neuron: Neuron talks to an effector cell across a synapse.
> effector cell: Does something, may be a muscle cell, or an endocrince cell, etc...
> Note the sensory neurons, interneurons, and motor neurons are part of the nervous system
> > but the sensory and effector cells do not need to be part of the nervous system

Knee-Jerk Reaction
> Fig. 48.3, knee-jerk reaction
> An example of information flow in vertebrates
> Stretch receptors in muscle organs; sense tension or release of tension
> > > Talks to sensory neuron across a synapse, IPSP or EPSP
> > sensory neurons; synapse from sensory cell onto this sensory neuron
> > > cell body of neuron in dorsal ganglion near spinal column
> > > enters dorsal side of spinal cord
> > > synapses with a motor neuron, and often with interneuron in spine
> > interneuron
> > > may be mostly in spine
> > > note many connections; gets information from sensory neuron
> > > missing from figure are connections to brain, etc...

motor neuron
        cell body in spine
        gets information from either sensory neuron or interneuron
        axon leaves ventral side of spinal cord
        may have stimulatory or inhibitor effects on effector cell
    effector cell
        in this case a muscle cell in a muscle organ
        muscle cell getting information from motor neuron
        may get an IPSP or an EPSP

Neural Circuits

Types of nerve circuits
        Fig. 35.5 (Gould and Keeton, 1996) neural_paths.jpg
Information all carried by one neuron with no sensory cell.
        So neuron is both sensory and motor cell, may still need effector cell.
Information from sensory cell, to neuron
        neuron direct to effector cell.
Information transduced across multiple cells. As in knee-jerk response.
information can go from single neuron to many postsynaptic neurons
        This spreads information out. Ex: information from eye to brain
        So branching is important
information from several neurons go to single postsynaptic neuron
        this concentrates information for summation/comparison/integration
        note use of stimulatory and inhibitory synapses
        ex: sensory information concentrated on one motor neuron
information can also flow in an apparently circular path
        ex: nerve net, as seen in hydra, as associated with our intestines
So wish to study how information flows, to try to understand how information is used

Studies with *Aplysia*

A model system for study of information flow...
        want to identify all cells involved in siphon withdrawal response
Example of *Aplysia*   (Gould et al., 1996. pgs. 996-997)
Fig. 35.6, (Gould etal., 1996) Aplysia_1.jpg
        A sea slug. Eric Kandel studied it.
        Just 2000 neurons in abdominal ganglion
                note L7 cell, entering nerves of many cells
                this is a SIMPLE system compared to many others, so good for a start
        large neurons so easy to monitor potentials as can impale with electrodes
Fig. 48.14, synaptic terminals
        a lot of them on just one cell, the L7 cell; a motor neuron

Fig. 35.7, (Gould etal., 1996) Aplysia_2.jpg
      Siphon withdrawal behavior; can use to study learning
      can desensitize, by repeated light touches.
          so will not withdraw siphon to water currents
      or can sensitize
          so will be withdrawn when danger occurs
Fig. 48.31, (Campbell and Reece, 2005) learning
      Could work out location of:
          sensory cell, sensory neuron, interneuron, motor neuron, siphon muscles
So this relatively simple system allows study of information flow and of learning
      that can occur. A nice example of the use of a model system.

Varieties of Nervous Systems
      Fig. 49.2, diversity of nervous systems in animals
      Note neural net of hydra, circular info flow?
      glanglia and nerve cords
          as seen in planaria, leech, insects
          cell bodies typically in ganglia, axons with myelin sheaths often in nerve cords
Cephalization; accumulation of nervous system elements in anterior end of body
      This can lead to what we call a brain. Contrast with sea star or hydra.
      cephalization is seen nicely in squids and chordates, and other animal groups
      Fig. 35.13, (Gould etal., 1996) worm_brain.jpg
          note earthworm ganglia is its brain, and the digestive tract goes through it!
      Allows comparison of information, for pattern recognition, etc...

Vertebrate Nervous System
      Structure of vertebrate nervous system
          Fig. 49.4, vertebrate nervous system
          CNS and PNS are organ systems,
             so more than just nervous tissue, consider other tissues needed...
          nervous tissue extends into many major organs as a part of them...
      central nervous system (CNS)
          Brain; obviously cephalized
          Spinal Cord; surrounded by supportive connective tissue/structures
          does integration, and comparisons
      Peripheral nervous system (PNS)
          cranial and spinal nerves, and ganglia
          carries signals to/from CNS, mostly carries information with little signal integration

Peripheral Nervous System
    Fig. 49.7, peripheral nervous system
    Several divisions, this figure shows the major groups to learn...
    will focus on efferent systems, to get information mainly from CNS
    Somatic
        connects to voluntary muscles
    Autonomic has three divisions...
        Parasympathetic division: P= placid, relaxing,
                gives lethargic feeling after a big meal
                Note exception at base of spine, no lethargy there...
        Sympathetic division: S = stimulate, "fight or flight"
                diverts blood flow to muscles, cuts it off of digestive tract
                and also makes a connection at the base...
            note effects on effector organs to prepare for response to threat
        Enteric division
            a network of neurons associated with digestive system
            often cross connected with the above two
            parts of this are somewhat similar to a nerve-net...

Comparison of Parasympathetic vs. Sympathetic Divisions
    Fig. 49.8, Parasympathetic vs. Sympathetic divisions
        Both have 2 neurons to effector cells from spine
        note sympathetic has ganglia along spine and long motor neurons
        parasympathetic has long interneuron and shorter motor neurons
    Fig. 35.35, (Gould etal., 1996) autonomic_NS.jpg
    note sympathetic has unique connections to
        adrenal medulla, note only one neuron in this pathway
                the medulla is derived from the second neuron
        uses epinephrine and norepinephrine as neurotransmitter,
                just like adrenal medulla

Central Nervous System
Vertebrate brain will be our model
General structure
    Fig. 49.5, ventricles, white and gray matter
    gray matter is mainly cell bodies and synapses
    white matter is mainly axons with sheaths
    ventricles are fluid filled regions, extend into spinal cord
        another body cavity... from chordate's dorsal hollow nerve cord...

General regions: Hindbrain, midbrain, forebrain
      Fig. 48.20, (Campbell and Reece, 2002) brain regions
      Hindbrain
            includes pons, medulla oblongata, and cerebellum
            carries out many autonomic functions
      Midbrain
            sensory information integration and sorting for sending to forebrain
      Forebrain
            includes cerebrum, thalamus, and hypothalamus, etc...
            Note, pituitary is part from the brain and part from the roof of mouth
               so parts of nervous system can also be endocrine in function
            Functions: Think about it!

Note development of human brain, focus on three regions (don't worry about the details here)
      Fig. 49.9, development of brain
      Starts linear, and becomes folded with development
Note how this compares to other mature animal brains
      Fig. 41.2, (Purves etal., 1998) animal_brains.jpg
      note expansion of forebrain
Contrast with how the cerebellum is very similar in proportion to brain for many animals...
      Fig. 18.15 Mammalian brains (Hildebrand and Goslow, 2001)
Note cerebellum convoluted in goose, not in frog or turtle...
      Fig. 18.14 Tetrapod brains (Hildebrand and Goslow, 2001)
      Fig. 49.14, bird and human brains
      birds are highly intelligent, but lack a convoluted neocortex
Midbrain and hindbrain are more conserved than forebrain.
      Fig. 18.13 Fish brains (Hildebrand and Goslow, 2001)

Male brains vs. female brains
      (based on paper by Cahill, 2005)
      Fig. 48.x1, (Campbell and Reece, 2002) MRI use
      Can use MRI to monitor brain structures
      Human male and female brains found to differ in a few specific ways.
            Similar in major ways, though different in average overall size
      Prevous to the 1960s, was assumed that there were major gender differences
            assumed to be based in the hypothalamus, but not supported by studies.
      Use PET and fMRI techniques to study human brains.
            Cahill 2005 sex diff.jpg
      Female has
            densier, bulkier frontal cortex, this ia areas of "higher" cognitive functions
            more developed limbis sytems, associated with emotional responses
            higher neural density in temporal lobe cortex, associated with language

Male has
>>larger parietal cortex, used in spatial relations
>>larger and higher neural density in amygdala, related to responses to stress
>>greater number of serotonin receptors, and higher serotonin production
>>larger hippocampus, another area related to stress responses
>>>also associated with spatial memory and navigation
When showed an emotion packed film segment monitored amygdala use
>>Cahill 2005 sex diff 2.jpg
>>Females used left side of amygdala,
>>males used right side of amygdala
>>>so same information processed differently?
>>Used propronolol to inhibit the actvity of the amygdala,
>>>it is an adrenaline inhibitor, a beta blocker
>>>In males this led to loss of the gist of memory,
>>>in females it led to loss of precise details
So sex differences in brains do occur. But no clear link of this to success in sciences.
>>(As was suggested by Larry Summers, president of Harvard in previous years...)

Maternal brain   (based on Kinsley etal., 2006)
>Kinsley etal 2006 Maternal Brain pg 75.jpg
>Several regions of brain thought to be altered:
>>hypothalamus (the medial preoptic area, mPOA), thalamus, cingulate cortex,
>>>nucleus accumbens, etc...
>Estrogen and progesterone are known to lead to change in aggression, sexuality, and
>maternal behavior.
>Prolactin known to be promotive of some maternal behaviors. As do endorphins.

>Some of these changes occur with hormones present during pregnancy.
>In other cases nursing/suckling induces nipples to release endorphins, and oxytocin.
>Human female gazes at her child, and it often activates a reward response in the brain.

>Estrogen and progesterone exposure leads to rise in size of some neurons in mPOA
>>region, and so alters and enhances the surface area of these neurons in
>>hippocampus.
>Oxytocin also stimulates changes in hippocampus.
>Hypothesis is that changes in maternal behavior is based to some degree on changes in
>>neurons.
>>Kinsley etal 2006 pg 77 neurons.jpg
>>Shows change in neurons, and see much more branching and larger sized cells.
>Evidence from monkeys noted that father's brains also change with arrival of offspring.

Plugging into the brain

(based on Nicoleis etal., 2002)

brain_electronics.jpg (Nicoleis etal. 2002, pgs. 48-49)

Owl monkey, with electrodes wired to 100 areas of her brain

Can insert fine electrodes and monitor hundreds of neurons at once.

Then have animal do task and monitor neurons.

Select those neurons which show change in activity with the desired behavior.

Can use this to create a model and predict from action potential data

what action would have been done.

Only 100s of neurons needed to do this well.

Can also show a cursor on a screen and train a monkey to think of moving the cursor to move it over a target and create a model which will use that pattern of neuron activity to drive the cursor accordingly.

There have been reports of paralyzed humans who can alter their EEG (electroenchephalogram) patterns and so use this change to select a letter from a screen and so write messages.

Fig. 48.22 (Campbell and Reece, 2002) EEG example

Use of magnetic fields to stimulate the brain

= transcranial magnetic stimulation (TMS).   (George, 2003)

It can alter alterness,   fight fatigue,   ease depression.

Funded by DARPA (Defense Advanced Research Projects Agency).

Transcranial_magnetic_stimulation.jpg (George 2003; pg. 69)

Field strengths used are 1.5 tesla.

(about 10000 greater than the Earth's magnetic field).

Pulses are given in 1 KHz range.

Induces electrical currents in neurons.

Field strength drops off drastically over 2-3 cm into body tissues.

so only stimulate surface areas of the brain.

Pattern of pulses used can give different effects.

Example. Can knock out speech centers for a short time.

Or can relieve or enhance depression.

May also alter memmory or learning?

In future it may be possible to have deeper penetration,

so could activate pleasure centers of the brain...

Antidepressant effects have been observed.

TMS report to have led to about 8 seizures, so high intensity or frequent use can be dangerous.

Summary

Information passing, processing, and comparison is a function of nervous system

Results in a rapid system for control of internal systems, reponses to environment.

Adaptive for animals that must act quickly. I.e. predators, prey...

Is capable of being changed over time, and with experiences...

Also see information passing in other multicellular lineages:

plants, fungi, some large algae...   so have analogous systems...

Objectives:

Be able to describe the differences between a sensory neuron, an interneuron, and a motor neuron in terms of their functions and connections. The roles of these in both the human knee-jerk and *Aplysia* siphon-withdrawal reflex arcs are good examples to work through in this context. Are only neuron cells involved in information transfer, or are other cells also involved?

Contrast both the structure and the antagonistic effects of the parasympathetic and sympathetic divisions in terms of the classical "flight or fight" response. For example: Which division has ganglia closer to the spinal cord? What are effects each system has when activated?

Be able to describe the basic organization of the vertebrate nervous system down to the level of divisions, and be able to name specific areas in, and functions of the forebrain, midbrain, and hindbrain of the vertebrate brain. Describe how the nervous system functions to compare and integrate external stimuli, its role in helping to maintain internal homeostasis, as well as its role in planning. Which areas of the CNS are most ancestral and which are most derived and modified in our lineage?

Describe several methods of monitoring brain activity. Is the brain static or malleable? Be able to give examples to illustrate the reasoning behind your answer.

For review see self-quiz questions #2, 3, and 5 of chapter 49.

Needed overheads and items:

Fig. 48.3, sensory, integration, response in neurons
Fig. 49.3, knee-jerk reaction
Fig. 35.5, (Gould and Keeton, 1996) neural_paths.jpg
Fig. 35.6, (Gould etal., 1996) Aplysia_1.jpg
Fig. 48.14, synaptic terminals
Fig. 35.7, (Gould etal., 1996) Aplysia_2.jpg
Fig. 48.31, (Campbell and Reece, 2005) Sea Hare Learning
Fig. 49.2, diversity of nervous systems in animals
Fig. 35.13, (Gould etal., 1996) worm_brain.jpg
Fig. 49.4, vertebrate nervous system
Fig. 49.7, peripheral nervous system
Fig. 49.8, Parasympathetic vs. Sympathetic divisions
Fig. 35.35, (Gould etal., 1996) autonomic_NS.jpg
Fig. 49.5, ventricles, white and gray matter
Fig. 48.20, (Campbell and Reece, 2002) brain regions
Fig. 49.9, development of brain
Fig. 41.2, (Purves etal., 1998) animal_brains.jpg
Fig. 18.15 Mammalian brains (Hildebrand and Goslow, 2001)
Fig. 18.14 Tetrapod brains (Hildebrand and Goslow, 2001)
Fig. 49.14, bird versus human brains
Fig. 18.13 Fish brains (Hildebrand and Goslow, 2001)
Fig. 48.x1, (Campbell and Reece, 2002) MRI
Cahill 2005 sex diff.jpg
Cahill 2005 sex diff 2.jpg
Kinsley etal 2006 maternal brain pg 75.jpg
Kinsley etal 2006 pg 77 neurons.jpg
brain_electronics.jpg (Nicoleis etal. 2002, pgs. 48-49)
Fig. 48.22 (Campbell and Reece, 2002) EEG example
Transcranial_magnetic_stimulation.jpg (George 2003, pg. 69)

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Purves W.K., G. H. Orians, H.C. Heller, D. Sadava- 1998-Life: The science of biology. 5th edition. Fig. 41.2. Sinauer Associates, Inc. Sunderland, MA.

Related issues:

For more on the **evolution** of the **central nervous system** and the **brain** see:

> Balter M- 2007-Brain evolution studies go micro- Science 315: (3/2) 1208-1211
> MacNeilage P.F., L.J. Rogers, G. Vallortigara- 2009-Origins of the left & right brain- Scientific American 301: (#1, July) 60-67

Here are some articles that deal with various aspects of **brain development** and **plasticity**.

> Akil H., S. Brenner, E. Kandel, K.S. Dendler, M-C. King, E. Scolnick, J.D. Watson, H.Y. Zoghbi- 2010-The future of psychiatric research: genomes and neural circuits- Science 327: (#5973, 3/26) 1580-1581
> Agulhon C., T.A. Fiacco, K.D. McCarthy- 2010-Hippocampal short- and long-term plasticity are not modulated by astrocyte Ca^{2+} signaling- Science 327: (#5970, 3/5) 1250-1254
> Grove E.A- 2008-Organizing the source of memory- Science 319: (#5861, 1/18) 288-289
> Manganas L.N., X. Zhang, Y. Li, R.D. Hazel, S.D. Smith, M.E. Wagshul, F. Henn, H. Benveniste, P.M. Djurќ, G. Enikolopov, M. Maletić-Savatić- 2007-Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain- Science 318: (#5852, 11/9) 980-985
> Merkle F.T., Z. Mizadeh, A. Alvarez-Buylla- 2007-Mosaic organization of neural stem cells in the adult brain- Science 317: (#5836, 7/20) 381-384
> Southwell D.G., R.C. Froemke, A. Alvarez-Buylla, M.P. Stryker, S.P. Gandhi- 2010- Cortical plasticity induced by inhibitory neuron transplantation- Science 327: (#5969, 2/26) 1145-1148

For more on **neural circuits,** and how they connect, their uses, and their speed of operation see:

> Bonifazi P., M. Goldin, M.A. Picardo, J.A. Catani, G. Bianconi, A. Represa, Y. Ben-Ari, R. Cossart- 2009-GABAergic hub neurons orchestrate synchrony in developing hippocampal networks- Science 326: (#5958, 12/4) 1419-1424
> Ecker A.S., P. Berens, G.A. Keliris, M. Bethge, N.K. Logothetis, A.S. Tolias- 2010- Decorrelated neuronal firing in cortical microcircuits- Science 327: (#5965, 1/29) 584-587
> Fields R.D- 2009-New culprits in chronic pain- Scientific American 301: (#5, Nov.) 50-57
> Schlegel T., S. Schuster- 2008-Small circuits for large tastes: High-speed decision-making in archer fish- Science 319: (#5859, 1/4) 104-106
> Wilson R.I- 2007-Neural circuits underlying chemical perception- Science 318: (#5850, 10/26) 584-585
> Womelsdorf T., J-M. Schoffelen, R. Oostenveld, W. Singer, R. Desimone, A.K. Engel, P. Fries- 2007-Modulation of neuronal interactions through neuronal synchronization- Science 316: (6/15) 1609-1612

And for those with **perfect pitch**....

Balter M- 2007-Study of music and the minds hits a high note in Montreal- Science 315: (2/9) 758-759

The connection of the brain to electronics (*The Matrix* anyone?) has practical uses in **neural implants**.

Miller G- 2008-Engineering a fix for broken nervous systems- Science 322: (#5903, 11/7) 847

Stix G- 2008-Jacking into the brain- Scientific American 299: (#5, Nov) 56-61

There have been reports of specific neurons in mammalian brains being associated with very specific functions, these are sometimes called dedicated **neural circuits**. For instance one such circuit is associated with memories of fear, and removal of it removes fear conditioning. Another study finds neural circuits associated with feelings of moral disgust, etc...

Chapman H.A., D.A. Kim, J.M. Susskind, H.K. Anderson- 2009-In bad taste: Evidence for the oral origins of moral disgust- Science 323: (#5918, 2/27) 1222-1226

Han J.H., S.A. Kushner, A.P. Yiu, H-L. Hsiang, T. Buck, A. Waisman, B. Bantempi, R.L. Neve, P.W. Frankland, S.A. Josselyn- 2009-Selective erasure of a fear memory- Science 323: (#5920, 3/13) 1492-1496

Lieberman M.D., N.I. Eisenberger- 2009-Pains and pleasure of social life- Science 323: (#5916, 2/13) 890-891

For more on uses of **transcranial magnetic stimulation** see:

Allen E.A., B.N. Pasley, T. Duong, R.D. Freeman- 2007-Transcranial magnetic stimulation elicits coupled neural and hemodynamic consequences- Science 317: (#5846, 9/28) 1918-1921

Jones L.S- 2007-The ethics of transcranial magnetic stimulation- Science 315: (3/23) 1663

Miller G- 2007-Uncovering the magic in magnetic brain stimulation- Science 317: (#5846, 9/28) 1846

Well, the bad news is in.... the human mind can not handle more than two sets of inputs at a time. Only two sets, because we only have **two hemispheres** in our brain and they can divide the work between them.   **Multitaskers** be warned!

Charron S., E. Koechlin- 2010-Divided representation of concurrent goals in the human frontal lobes- Science 328: (#5976, 4/16) 360-363

Of course, we are mainly interested in the brain due to issues concerning the **mind**, **consciousness**, and **memory**. But what is the physical basis of these? Here are articles that try to take a stab at these issues...

Alkire M.T., A.G. Hudetz, G. Tononi- 2008-Consciousness and anesthesia- Science 322: (#5903, 11/7) 876-880

Balter M- 2010-Did working memory spark creative culture?- Science 328: (#5975, 4/9) 160-163

Depue B.E., T. Curran, M.T. Banich- 2007-Prefrontal regions orchestrate suppression of emotional memories via a two-phase process- Science 317: (#5835, 7/13) 215-219

Ehrsson H.H- 2007-The experimental induction of out-of-body experiences- Science 317: (#5841, 8/24) 1048

Euston D.R., M. Tatsuno, B.L. McNaughton- 2007-Fast-forward play back of recent memory sequences in prefrontal cortex during sleep- Science 318: (#5853, 11/16) 1147-1150

Han J-H., S.A. Kushner, A.P. Yiu, C.J. Cole, A. Matynia, R. A. Brown, R.L. Neve, J.F. Guzowski, A.J. Silva, S.A. Josselyn- 2007-Neuronal competition and selection during memory formation- Science 316: (4/20) 457-460

Lenggenhager B., T. Tadi, T. Metzinger, O. Blanke- 2007-Video *ergo sum*: Manipulating bodily self-consciousness- Science 317: (#5841, 8/24) 1096-1099

McHugh T.J., M.W. Jones, J.J. Quinn, N. Balthasar, R. Coppari, J.K. Elmquist, B.B. Lowell, M.S. Fanselow, M.A. Wilson, S. Tonegawa- 2007-Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network- Science 317: (#5834, 7/6) 94-99

Miller G- 2007-Out-of-body experiences enter the laboratory- Science 317: (#5841, 8/24) 1020-1021

Pessiglione M., L. Schmidt, B. Draganski, R. Kalisch, H. Lau, R.J. Donlan, C.D. Frith- 2007-How the brain translates money into force: A neuroimaging study of subliminal motivation- Science 316: (5/11) 904-906

Raichle M.E- 2010-The brain's dark energy- Scientific American 302: (#3, March) 44-49

Rawashdeh O., N. Hernandez de Borsetti, G. Roman, G.M. Cahill- 2007-Melatonin suppresses night time memory formation in zebrafish- Science 318: (#5853, 11/16) 1144-1146

Reijmers L.G., B.L. Perkins, N. Matsuo, M. Mayford- 2007-Localization of a stable neural correlate of associative memory- Science 317: (#5842, 8/31) 1230-1233

Rosenbaum R.S., D.T. Stuss, B. Levine, E. Tulving- 2007-Theory of mind is independent of episodic memory- Science 318: (#5854, 11/23) 1257

Schurger A., F. Pereira, A. Treisman, J.D. Cohen- 2010-Reproducibility distinguishes conscious from nonconscious neural representations- Science 327: (#5961, 1/1) 97-99

Schwarzkopf D.S., G. Rees- 2010-Brain activity to rely on?- Science 327: (#5961, 1/1) 43-44

Shema R., T.C. Sacktor, Y. Dudai- 2007-Rapid erasure of long-term memory
associations in the cortex by an inhibitor of PKMζ- Science 317: (#5840, 8/17)
951-953

Tsien J.Z- 2007-The memory code- Scientific American 297: (#1, July) 52-59

Wang H-P., D. Spencer, J-M. Fellows, T.J. Dejnowski- 2010-Synchrony of thalamocortical
inputs maximizes cortical reliability- Science 328: (#5974, 4/2) 106-109

This article relates activities in certain regions of the **brain** with certain types of **mental illnesses**.

Insel T.R- 2010-Faulty circuits- Scientific American 302: (#4, April) 44-51

In the new field of **optogenetics** light-sensitive ion channels are introduced by viral infection, when put into neurons this allows light pulses to be used to rapidly alter the induction of action potentials in selected neurons.

Miesenböck G- 2008-Lighting up the brain- Scientific American 299: (#4, Oct.) 52-59

Misenböck G- 2009-The optogentic catechism- Science 326: (#5951, 10/16) 395-399

Here is an article that notes the limits of **fMRI** and its use in studying neural circuits.

Friston K.J- 2009-Nodalities, modes, and models in functional neural imaging- Science 326:
(#5951, 10/16) 399-403

This study finds a neurological basis for the **placebo** affect:

Eippert F., J. Finsterbusch, U. Bingel, C. Büchel- 2009-Direct evidence for spinal cord
involvement in placebo analgesia- Science 326: (#5951, 10/16) 404

BIO 107   2010
Day 13, Lecture 30, Title: Senses I: Basic human senses.

Text Readings: Campbell etal. (2008), pgs. 1087-1105.

Topics to cover:

>   **Sensory Overview**
>   **Naked Neurons as Sensory Cells**
>   **Non-Neuron Sensory Cells**
>>   **Taste**
>>   **Hearing**
>>   **Sight**

Sensory Overview
>   Relate to signal, reception, transduction, response systems
>   Same concepts as seen in past, but now across animal systems... so multicellular
>>   will focus here mainly on what happens in sensory cells
>   Fig. 48.3, Overview of Vertebrate nervous system
>   receptors
>>   signal to noise ratio must be high, otherwise signals are not sensed
>>>   signal must exceed background noise
>>>   must be a receptor, or do not get the message...
>>   need for signal information amplification for rapid response
>>>   note information, not original signal, is amplified
>>>   if not done, then response will be slower...
>   signal information transduction
>>   here can consider events in sensory cell
>>   or how signal information moves through organism
>   integration/response
>>   integration often in nervous system
>>   response involves motor neurons and effector cells...

Naked Neurons as Sensory Cells
>   Some human sensory cells are neurons..
Use dendritic neuron endings as sites of receptors
>   smell
>>   Fig. 50.15, olfaction in humans
>>   receptors are on modified neurons exposed to air, note sensory cilia
>>   often are ligand-gated ion channels
>>   various genes code for distinct binding sites, so different odors detected

pain

   Fig. 50.3, sensory receptors in human skin

   note pain receptors are naked dendrites

     respond to damage,

     pain in part via histamines from damaged cells, or heat, pressure

   Note, these or often non-mylinated neurons,

     what does this do to speed of transmission?

     Relate hitting your hand with a hammer, to realization, to feeling of pain.

Non-Neuron Sensory Cells

   Many sensory cells are not derived from neurons. Instead are from other tissue types.

   In this case they have to communicate with sensory neurons via synapses.

Taste

First the old view (Tonosaki et al., 1988)

   Fig. 49.14, (Campbell and Reece, 2005) taste receptors and action potential frequency

   note the cells in taste buds

   receptor for sugar is one type, others for other items

     Note this is a G-protein coupled receptor

   signal transduction in sensory cell once receptor activated

     note use of cAMP, protein kinase...

   leads to change in channel activity, and so change in membrane potential

     in this case depolarization by stopping K^+ exit

   eventually voltage-sensitive calcium ion channels activated

     leads to a calcium ion spike in cytosol, induces exocytosis

   exocytosis of vesicles containing neurotransmitters

     this is the response of this sensory cell, but not the response of the organism...

   induces sensory neuron to fire off more action potentials

     note this changes the frequency of action potential firing,

      not the nature of each action potential.

   This puts receptor and neurotransmitter release all in one sensory cell

   Notice how similar this is to what we saw in presynaptic neuron's axon terminal.

     unity in life...

Now the new model   (Remember these are models, so this may all change...)

   This part is from text, newer vision of taste bud

   Fig. 50.13, taste reception and transduction

   A few changes here, but some things the same,

     GPCR, G protein, the same.

     Changes

      Phospholipase C activation, cleaves a lipid

      this makes IP_3, acts as a secondary messenger

      IP_3 detected by Ca^{+2} channel in ER

      Activation of this channel releases Ca^{+2} to cytosol from the ER lumen

      The rise in IP_3 and Ca^{+2} concentrations activates a Na^+ channel

      The movement of Na^+ alters the plasma membrane potential

From here the text does not connect this to the sensory neuron
Recent work makes this connection: (see handout)
     Taste bud 1.jpg
     Taste bud 2.jpg
     Roper (2007) notes that receptor is in one cell,
          and serotonin (neurotransmitter) is released from another.
          ATP is used to signal from one cell to the other.
     ATP is released via a hemichannel, a structure similar to a gap junction
            (Huang et al. 2007)
          This structure is activated by a change in the membrane potential.
     Tight junctions in the taste bud create groups of cells that signal each other.
          There is also ATPase secreted to remove the secreted ATP.
     So receptor is in one cell, information of signal reception is processed through
          several cells, until one cell in the group of taste bud cells secretes
          neurotransmitter onto their sensory neuron.

Hearing
Human ear parts
Fig. 50.8, human ear structures
Note: pinna, auditory canal leading to tympanic membrane
     note how these structures concentrates vibrations, for amplification
bones of middle ear, malleus, incus, stapes
     recall, these are modified from pharyngeal slits (we are chordates)
     note area of tympanic membrane versus area of oval window, for amplification
oval window, leads to vestibular canal which is fluid filled
     vestibular canal extends through cochlea
     wraps back on lower side as tympanic canal to round window, in cochlea
     fluid filling, so incompressible and transmits vibrations well
Cochlea
     Has the vestibular and tympanic canals inside of it, coiled around
     Note cochlear duct, fluid filled, basilar membrane, tectorial membrane, hair cells,
          and sensory neurons
          95% of afferent neurons from inner hair cells just 5% from outer hair cells
     If unfold it see upper vestibular canal continuous with lower tympanic canal
          Fig. 50.10, Cochlea pitch and vibration
     Note Cochlear duct in between these two canals, so vibrations influence it
     Vibration of basilar membrane in cochlea will give stimulus, allow pitch detection
          Fig. 50.8, cochlear duct
Hair cells and mechanosensing of vibrations
     In cochlear duct are hair cells
     Fig. 50.9, mechanoreceptors in hair cells
     Draw on board: (Walcott, 2002)
     Cilia of hair cells bump into tectorial membrane
          due to vibration of basilar membrane
     endolymph has, in cochlear duct

high [K^+], low [Na^+], low [Ca^{+2}], odd for an extracellular fluid...

cell cytosol is -80 mV more negative relative to endolymph

tight junctions by cells lining cavities, so hold ions and potentials

perilymph in vestibular and tympanic canals has

low [K^+], high [Na^+], high [Ca^{+2}], more typical for extracellular fluid...

Note, these canals and this duct are internal body cavities... coeloms

inner hair cell straddles these two lymphs

cilia in endolymph, base of cell exposed to perilymph

with vibration K^+ ion channels open

K^+ enters cell and depolarizes it as K^+ enters

this alters membrane potential

results in voltage sensitive Ca^{+2} channels opening at other end of cell

cell cytosol is -45 mV relative to perilymph

so calcium ions enter cell, produce calcium ion spike in cytosol

and this stimulates exocytosis of vesicles

this releases neurotransmitter at synapse with sensory neruon

transmitter stimulates AP in neuron

Δstimulus amplitude leads to change in action potential frequency

Different receptor, and different flow of K^+ due to odd endolymph,

but membrane potential depolarization, calcium ion spike, exocytosis

so much of this is similar to other systems we have already seen...

Sight

Human eye structures

Fig. 49.18, human eye

note: retina, choroid, optic nerve, lens, vitreous humor, aqueous humor, cornea,
pupil, iris, suspensory ligaments

Retinal structures

Fig. 49.23, vertebrate retina, cellular level

note orientation in human eye

photoreceptor cells (rods and cones) are sensory cells, nervous tissue

horizontal and bipolar cells act as sensory neruons

note only bipolar cells connect to ganglion cells

horizontal cells influence activity of bipolar cells

other neurons

Ganglion cells have axons that extend through optic nerve to brain

amacrine cells act to influence activity of ganglion cells

note lateral connections by horizontal and amacrine cells

        This allows for processing of signal information, pattern recognition

        For instance, it allows lateral inhibition,

            so edges about line look brighter and darker than expected

Rod and cone cells

    Fig. 49.20, rod cell parts

        cone = color, rod = B&W

        note membranous regions of disks,

        rhodopsin, in membranes of disks, two parts of it

            opsin = protein

            retinal is an organic molecule cofactor

    Fig. 49.15x, (Campbell and Reece, 2002) SEM of cells in retina

Rhodopsin changes with light absorption

    Fig. 50.20, retinal changes with light

        note change in shape with light, Δ retinal from cis to trans form occurs

        later retinal dissociates from opsin, but that is slow

        the retinal shift alters shape of rhodopsin,

            this will alter G-protein transducin's activity

        so rhodopsin is a G-protein coupled receptor...

Signal transduction in rod cell

    Fig. 50.21, rod cell signal transduction

    Note that transducin is a G-protein, so has GTPase activity.

    Transducin, binds Phosphodiesterase

    Phosphodiesterase cleaves cGMP (similar to cAMP but different base...)

    loss of cGMP results in closing of ligand-gated Na^+ channel

        one photon results in a million channels to close

            a good example of signal information amplification

    note Na^+ channel normally open (Walcott, 2002)

        allows Na^+ entry, and holds membrane potential in depolarized state

    With fewer Na^+ entering, get membrane hyperpolarization...

Fig. 50.22, synapes, rod cells and bipolar cells

    voltage sensitive Ca^{+2} channels are turned off,

    concentration of Ca^{+2} in cytosol drops

    fewer vesicles fuse at synapse

    less neurotransmitter released, in this case glutamate

    glutamate is an inhibitory neurotransmitter, induces IPSP in sensory neruon

        less inhibition, fewer IPSPs, and so rise in action potential firing seen

So here see ligand-gated Na^+ channel, voltage sensitive Ca^{+2} channels, vesicle fusion,

    once again many similar parts to what we saw in synapses

    But here the "switch" starts on, and signal reception turns it off

        releasing the sensory cell from inhibition

    So response by sensory cell is to lower neurotransmitter release

If time: pattern reception and optical illusions
        Our perceptions are often due to pattern recognition,
                not always a true representation of reality, focus on certain features...
                hopefully adaptive
        Water color effect, fill in space with color that is not there
                watercolor effect.jpg (Werner etal. 2007)
        Lateral inhibition
                Note connections of horizontal cells
                        relative to field of illuminated rod and cone cells
                        Fig. 49.23, (Campbell and Reece, 2005) vertebrate retina, cellular level
                Draw out effects across black/light border in terms of perceptions
        Examples
                Scintillating Luster.jpg (Werner etal. 2007)
                        Note where black rods meet center gray, lateral inhibition
                Ehrenstein figure.jpg (Werner etal. 2007)
                        note inserting ring removes effect
                Bright Color Patches.jpg (Werner etal. 2007)
                        center looks whiter than same white outer border
                Anomalous Brightness.jpg (Werner etal. 2007)
                        a similar example
                Anomalous Darkness Induction.jpg (Werner etal. 2007)
                        can also make center look darker than dark...
                Flashing anomalous Color Contrast.jpg (Werner etal. 2007)
                        center seems to flash as eye moves
                optic_illusion.jpg (Haseltine, 2003)
                        rotation appear to produce movements that do not exist

Objectives:

For an organism to sense an environmental cue what must it have? For it to respond, what must it do? Are all environmental signals able to be sensed by all organisms? When a signal is sensed by a mammal what happens to the frequency of action potentials that are sent through sensory neurons?

Be able to describe the important structures involved in the senses of taste, hearing and sight in humans. In each case be able to describe the receptor system, and the steps in signal transduction in the sensory cells involved that occur and how they influence action potential firing in an associated sensory neuron. For instance, how the change in shape in rhodopsin in the eye results in a change in the frequency in action potentials in a neighboring bipolar cell should be clear to you. Contrast the human sense systems of taste, hearing and sight in terms of the amount of processing or modification of the signal information that occurs before information is sent to the brain. If processing is done, is it done by the sensory cells, neurons, or by other structures? If given a diagram, be able to locate such structures and locate the sensory cells and the sensory neurons involved in human taste, hearing and sight (figures 50.8, 50.18, 50.23, and in the handout are good practice here). Consider how these senses differ in terms of the nature of the signal, the relative energy content of the signal, and the speed of change in the signal that can be detected.

Be able to compare and contrast the elements of signal transduction used in taste, hearing, and sight sensory cells to elements of signal transduction used at a neural synapse.

For review see self-quiz questions #3, 4, and 5 of this chapter.

Needed overheads and items:

Fig. 48.3, Overview of Vertebrate nervous system
Fig. 50.15, olfaction in humans
Fig. 50.3, sensory receptors in human skin
Fig. 49.14, (Campbell and Reece, 2005) taste receptors and AP frequency
Fig. 50.13, taste reception and transduction
taste bud 1.jpg
taste bud 2.jpg
Fig. 50.8, human ear structures
Fig. 50.10, pitch distinguishing
Fig. 50.8, human ear structures
Fig. 50.9, mechanoreceptors in hair cells
Fig. 50.18 human eye
Fig. 50.23, vertebrate retina, cellular level
Fig. 50.20, rod and cone cells
Fig. 49.15x, (Campbell and Reece, 2002) SEM of cells in retina
Fig. 50.20, retinal changes with light
Fig. 50.21, rod cell signal transduction
Fig. 50.22, synapes, rod cells and bipolar cells
Watercolor effect.jpg (Werner etal. 2007)
Fig. 49.23, (Campbell and Reece, 2005) vertebrate retina, cellular level
Scintillating Luster.jpg (Werner etal. 2007)
Ehrenstein figure.jpg (Werner etal. 2007)
Bright Color Patches.jpg (Werner etal. 2007)
Anomalous Brightness.jpg (Werner etal. 2007)
Anomalous Darkness Induction.jpg (Werner etal. 2007)
Flashing anomalous Color Contrast.jpg (Werner etal. 2007)
optic_illusion.jpg (Haseltine, 2003)

Handout:
Lecture 30 - Senses I - Handout.stm
        (Taste bud 1.jpg and Taste bud 2.jpg)
        Modified from
                Campbell and Reece (2005), fig. 49.14
                Campbell et al. (2008), fig. 50.13

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Sensory and Motor Mechanisms. Chapter 50. Pages 1087-1105. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

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Haseltine E- 2003-Round and Round; The brain's miserly habits can make your head spin. Discover 24: (#5) 88

Huang Y-J., Y. Maruyama, G. Dvoryanchikov, E. Pereira, N. Chaudhari, S.D. Roper- 2007-The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste bud- PNAS 104: (#15, Apr. 10) 6436-6441

Roper S.D- 2007-Signal transduction and information processing in mammalian taste bud- European Journal of Physiology 454: 759-776

Tonosaki K., M. Funakoshi- 1988-Cyclic nucleotides may mediate taste transduction- Nature 331: (Jan. 28) 354-356

Walcott C- 2002-Lecture 29: Sensory Transduction. Introductory Biology (BIOG 101) Cornell University.

Werner J.S., B. Pinna, L. Spillmann- 2007-Illusory Color & the Brain- Scientific American 296: (#3, March) 90-95

Related issues:

Here are some interesting articles dealing with **sight** in various animals: Its evolution, how it is used, and one article on making implants to replace eyes.

Billock V.A., B.H. Tsou- 2010-Seeing forbidden colors- Scientific American 302: (#2, Feb.) 72-77

Fernald R.D- 2006-Casting a genetic light on the evolution of eyes- Science 313: (9/29) 1914-1918

Goldsmith T.H- 2006-What birds see- Scientific American 295: (#1, July) 68-75

Koretz J.F., G.H. Handelman- 1998-How the human eye focuses- from July 1998 of Scientific American. Reprinted in pgs 14-21 of Science's Vision: The mechanics of sight, by Scientific American.

Lazzaro J- 2004-Artificial vision and the "kite and key" experiment- Analog: Science Fiction Science Fact (July/August) 58-68

Levy O., L. Appelbaum, W. Leggat, Y. Gothitf, D.C. Hayword, D.J. Miller, O. Hoegh-Guldberg- 2007-Light-responsive cryptochromes from a simple multicellular animal, the coral *Acropora millepora*- Science 318: (#5849, 10/19) 467-470

Marx J- 2007-High-risk glaucoma gene found in Nordic studies- Science 317: (#5839, 8/10) 735

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Here chapters in a text that deals with the evolution of **hearing** in fish and mammals, amongst other matters:

Benton M.J- 2000-The evolution of fishes after the Devonian- Chapter 7, pgs. 157-185, in- Vertebrate Palaeontology. 2nd edition. Blackwell Science. Oxford, U.K.

Benton M.J- 2000-The mammals- Chapter 10, pgs. 287-362, in- Vertebrate Palaeontology. 2nd edition. Blackwell Science. Oxford, U.K.

This is a study of **hearing** in bats, and their use of **echolocation**.

Yovel Y., B. Falk, C.F. Moss, N. Ulanovsky- 2010-Optimal localization by pointing off axis- Science 327: (#5966, 2/5) 701-704

This article touches on aspects of **hearing** in whales, and other features of marine mammals.

> Wong K- 2002-The mammals that conquered the seas- Scientific American 286: (#5)
> 70-79

This article describes sounds made by fish, implying they can **hear** those sounds as well.

> Parmentier E., O. Colleye, M.L. Fine, B. Frédérich, P. Vandewalle, A. Herrel- 2007-
> Sound production in the clown fish *Amphiprion clarkii*- Science 316: (5/18)
> 1006

The use of **electronic implants** to help with **hearing** and **balance** is being refined.

> Della Santina C.C- 2010-Regaining balance with bionic ears- Scientific American 302: (#4,
> April) 68-71

The senses of **taste** and **smell** are dealt with in the following: The komodo dragon is said to have a very developed sense of smell. Our tongues have been suggested to taste carbon dioxide. They have found taste receptors not just in our mouths, but also in our air passages. If we understand how taste works, then it should be possible to alter the taste of food, so this is an area much studied by food scientists.

> Ballantyne C- 2007-Losing scents- Scientific American 297: (#6, Dec.) 26-28
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> Kinnamon S.C., S.D. Reynolds- 2009-Using taste to clear the air(ways)- Science 325:
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> Pennisi E- 2006-Honey bee genome illuminates insect evolution and social behavior-
> Science 314: (10/27) 578-579
> Shah A.S., Y. Ben-Shahar, T.O. Moninger, J.N. Kline, M.J. Welsh- 2009-Motile cilia of
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> Shepherd G.M- 1988-Chemical senses- Chapter 11, pgs. 222-246, in Neurobiology,2nd
> edition. Oxford University Press, N.Y.
> Smith D.V., R.F. Margolskee- 2001-Making sense of taste- Scientific American 284:
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> Wenner M- 2008-Magnifying taste- Scientific American 299: (#2, Aug.) 96-99

How influential are our senses? When we feel warm, and we feel warmth towards someone, it turns out that a similar set of neuronal circuits are used. Thus our **sensory wiring** has some connection to our emotions.

Williams L.E., J.A. Bargh- 2008-Experiencing physical warmth promotes interpersonal warmth- Science 322: (#5901, 10/24) 606-607

Here is a report of **technology** that allows the **blind** to see, somewhat. It does it by sending signals to the tongue!

Kendrick M- 2009-Tasting the light- Scientific American 301: (#4, Oct.) 22-24

BIO 107   2010
Day 13, Lecture 31, Title: Senses II.
Text Readings: Campbell et al. (2008), pgs. 1087-1092, 1094-1096, 1099-1100.

Topics to cover:

>   **Limits of sensing**
>   **Diversity of what can be detected**
>   **Converting external information to internal information**
>   **Odd senses**
>> **Gravity**
>> **Pressure**
>> **Electrical Fields**
>> **Magnetic Fields**
>> **Thermal Sensing**
>> **Hearing in Insects**
>> **Insect sight**
>> **Fungal Touch**
>> **Plant Senses**

Limits of sensing
There is a lower limit to sensitivity; to what can be sensed....
>   Even if detect one photon, how much energy must it have?
>>   red photon vs. blue photon, blue easier to detect
>   note signal to noise requirements
>   Energy in Noise: One type of this "noise" is molecules randomly bumping into each other.
>>   How much energy (E) are in these bumps?
>> $E = kT$, Boltzman constant times degrees Kelvin
>>> $k = 1.38 \times 10^{-23}$ J/degrees K
>>> $T = 298$ degrees K
>>>   energy of background heat $= 3.84 \times 10^{-21}$ J
>>   chemical bonds must resist these energies to be stable
>   Energy in Signal: has to exceed above noise to be detected
>>   One photon of green light (550 nm)
>>> $E = h\nu$, Plank's constant times the frequency of light
>>>>   (recall $C = \lambda\nu$; so frequency is 5.45×10^{14} Hz for this color)
>>>> $(6.63 \times 10^{-34}$ J/Hz$)(5.45 \times 10^{14}$ Hz$) = 3.61 \times 10^{-19}$ J
>>>   So a green photon is 100X background noise energy, so can detect light
>>   Could calculate energy in other signals such as concentration gradients, etc...
Even if present, and of enough energy, not all signals are detected.
>   lack of receptors, lack of sensory mechanisms, energy of signal can be too low
>   energy of signal must be enough to cause a change in protein shape,
>>   so it must alter H-bonds or van der Waals interactions at least.

Diversity of what can be detected

      Various molecular mechanisms of detection

      Come back to mostly proteins acting as the detector

           any signal strong enough to alter the structure of a protein can be detected

      Fig. 42.1 (Purves etal., 1998) sensors.jpg

      can detect

           vibration, temperature, electrical fields, magnetic fields, gravity, light,

           concentration gradients, etc....

      note use of ion channels seen in much of above, involves changes in protein shape,

           voltage sensitive ion channels do this in response to membrane potential gradient

      it does not have to be ion channels, but many sensory systems do use them

           membrane potential changes spread quickly

           any slow response might use other types of receptors...?

           ex: in this figure the thermosensor is coupled to an enzyme...

               how fast does it work?

Converting external information to internal information

Not merely presence of signal can be detected, signal can have other features...

Information of intensity of signal, and even of rates of change

      In animals action potentials are steriotypical, same amplitude, same duration

           how to use them to carry signal information?

           Use frequency of action potentials, and pattern of firing

      Example; mechanoreceptor sends off more action potentials with faster displacement

           so this carries information that movement was fast or slow

           Fig. 7.9 (Ekert and Randall, 1978) velocity_detector.jpg

           so more rapid change in signal results in more APs

      Senses can detect rate of change of a signal...

Sensory adaptation

      Moving from dark room to bright sunshine

      Entering heated room from winter day, room seems hot

      Some receptors respond to changes in signals, not just signals,

      Fig. 7.10 (Eckert and Randall, 1978) tonic_phasic.jpg

      Phasic receptors, returns to baseline firing rate after initial stimulus change is over.

           So this detects change

      Tonic receptors, will continue with higher firing rate while stimulus is present,

           even if steady. So this detects presence.

      Nervous system must then integrate above and search for patterns to give perception

           with no change, the signal drops from our conscious perception

           Note the phasic receptors that induce action potentials only with change

           They turn off over time, and this illustrates sensory adaptation

                not just of nervous system but of the sensing cell and sensory neuron

                less response to a persisting signal

      So we are cued in to changes in signals, not just signals

Odd senses

Some examples of odder senses: Others exist we will not cover, or do not know about
The sensory systems of an organism determine what it experiences, and to what its
     responses should be adaptive.
Will cover just a few examples here...

Gravity
(We use our inner ear...) Consider how invertebrates detect gravity orientation
Statoliths are crystals in an invertebrates' internal body cavity
     Fig. 50.6, invertebrate statoliths
Fluid filled chamber, note use of hair cells
     bumping of statoliths induces mechanoreceptors in hair cells.
     Depends on difference in density of crystal versus fluid in chamber

Pressure
Pressure sensors (we have these in our skin and our circulatory system...)
lateral line system in fish
Fig. 50.12, fish lateral line
     note modification of what we saw in ear, hair cells
     water in line carries pressure waves
Fig. 4.23, Hair cells (Alberts etal. 1994)
     another mechanoreceptor system based on hair cells

Electrical Fields
(This is a sense we lack as organisms, but we have many voltage-sensitive ion channels...)
Electroreceptors   (Eckert etal., 1978)
Hair cells in fish modified to sense electrical fields
Positive charges into cell, depolarizes cell, induces more frequent action potentials
Positive charges out of cell, hyperpolarizes cell, represses action potential frequency
So action potential firing frequency is proportional to electrical field encountered
     with sensitivity down to the micro-volt range!
Fish use above to detect prey
     weak electric fish create electric fields, tail to head
     objects in field can interfere with current, and so be detected.
          Fig. 7_38 Electric sense in fish.jpg (Eckert and Randall, 1978)

Magnetic Fields
(Another sense we apparently lack...)
Earth's field is roughly 50 µT   (Hemmersbach etal., 1997)
Paramecium biaurelia, *Loxodes striatus*, *Tetrahymena thermophila*
These all may sense magnetic fields
These cells exposed to fields
 at 50 Hz, of 0.5-2.0 mT magnetic field.
 In response see a dose dependent increase in swimming velocity,
  and a rise in the amount of turning done.
Some suggestion of magnetic sense in pigeons for homing, mechanism still being examined
 pigeon magetic sense.jpg (Fig. 38.38, Gould and Keeton 1996)
 exposure to magnetic field alters direction traveled on cloudy days to find nest
Also suggestions of use of magnetic fields for their migration
 Fig. 50.5, whale migration

Thermal Sensing
(This is a sense we have in our skin and internally...)
Thermoreceptors   (Eckert etal., 1978)
Rattlesnakes can sense temperature differences
 Fig. 50.5, rattlesnake
 as small as 0.002 degrees C differences can be detected!!
 So can sense a mouse at 40 cm away if the mouse
  is 10 degrees C warmer than background
 Two sensor pits, so sterio-sensing for position information

Hearing in Insects
(A bit different from ours)
Hearing in insects, nocturnal moths
tympanic membrane, can be in legs or along sides of abdomen!
Fig. 50.7, tympanic membrane of insect
This connects sound to vibration perception
3 neurons per ear, two acoustic, one mechanical sensor (Walcott, 2003)
 The two acoustic neurons cover different ranges of sounds
 Mechanical sensor can monitor wing position, might muffle sound
  so insect can tell that a muffled sound does not mean source if far...
 can hear up to 100 KHz, the sonar range of bats
  Fig. 50.1, bat and moth
Can tell if sound is from above or below by comparison with change in wing position
 so must compare imput from mechanical sensor and acoustic sensors over time
If hear bat sonar in one side vs. other move away
If hear bat sonar loud in both, then dives for ground! Can mimic this with keys...
Note: (Corcoran et al., 2009) Some insects use a blast of sound to confuse bats!

Aside: mites infect moth ears

This makes it deaf
Generally one ear per moth is infected...
     70% of moths have infected ears, half right, half left, none in both
As enters lays down pheromone track
other mites entering follow first
This leaves one ear functional, otherwise moth is eatten by bat, and so mites die
     selection for survival of parasite that perserves life of host...

Insect sight
(Also a bit different from ours in structure and in range...)
Fig. 50.17, insect compound eye
extends to UV light range (Weiss, 1995)
Fig. 49.08x2, (Campbell and Reece, 2002) insect vision
     So what they see can differ from what we see..
     using butterflies
     found butterflies can be trained to look for
        nectar in certain colored flowers, all same shape
     uses butterflies to remove nectar from selected flowers based on their sensing

Fungal Touch
Sense of touch and shape is a type of thigmoism
Fungi thigmoisms influence how it grows   (Hoch, 2002)
Rust fungi, attach to surface of plant leaves and need to enter to get to cells in leaf
spores grow tubes on leaf surface, hyphae grow over surface
     Fig. 31.4, Fungal hyphae
     in danger of being washed, shaken, off unless enter leaf
     when get to stomatal opening fungus grows an appresorium to enter
structural of stomatal opening has a 0.6 - 1 μm external ridge about stoma pore
Using nanofabrication facility to form test surfaces/ridges
     grew fungi on test surfaces, and shape similar to stomatal opening induces
        appresoria formation..

Plant Senses
Obviously they have many senses, will just give a list here...
     some action potentials found, other intraorganismal transduction systems exist

Sense gravity, for upright shoots and downward roots
     Wayne etal. (1996)   review of plant gravity sensing
     Statolith model
        starch grains in root cap cells
     Falling of cell by 1 nm give $100000 > kT$
        stretches plasma membrane, tension put on proteins, may alter activity?

sense sunlight, obviously needed to position leaves, tell if in understory or not... etc.
     to tell if shaded or sunny

           sense red/far red light
           Stoma sense light to tell when to open or close
    sense odors
           for items from other plants, are they damaged?
           ethylene, carbon dioxide
    sense vibration and touch
           Venus fly trap, and sundew, etc...
    Even a sense of self
           Ex: maple leaved passion flower
           Fig. 6, (Gray, 1873) passion_flower_tendrils.jpg
           will not coil on self, insteads backs up, and raises over self
           so plants can distinguish self from non-self; they have a sense of self!

Sensing is a part of life. A living organism that can not receive signals from the environment is in trouble...

Objectives:

How does the energy in an environmental signal have to compare to the noise in the environment in order for the signal to be detected by an organism? What is the typical type of molecule that is used by life as signal receptors and what must happen to that molecule for a signal to be received?

Do senses allow a complete, accurate, and truthful perception of the environment by a species?

How does information about the intensity, or other features, of a signal get sent through the animal nervous system when each action potential is just like any other action potential?

What is the difference between a phasic receptor and a tonic receptor? What advantage do we get from having both types of receptor systems?

Be able to describe several different molecular mechanisms that might be used to receive an environmental signal. Be able to describe common features of how various types of signals can be sensed by life forms, and describe how such sensing is adaptive for the organism that has each sense.

For review see self-quiz questions #1 and 2 of chapter 50.

Needed overheads and items:

Fig. 42.1 (Purves etal., 1998) sensors.jpg
Fig. 7.9 (Eckert and Randall, 1978) velocity_detector.jpg
Fig. 7.10 (Eckert and Randall, 1978) tonic_phasic.jpg
Fig. 50.6, invertebrate statolith
Fig. 50.12, fish lateral line
Fig. 4.23, Hair cells (Alberts etal. 1994)
Fig. 7_38 Electric sense in fish.jpg (Eckert and Randall, 1978)
Fig. 38.38 (Gould and Keeton 1996) pigeon magetic sense.jpg
Fig. 50.5, whale migration
Fig. 50.5, rattlesnake
Fig. 50.7, tympanic membrane of insect
Fig. 50.1, bat and moth
Fig. 50.17, insect compound eye
Fig. 49.08x2, (Campbell and Reece, 2002) insect vision
Fig. 31.4, Fungal hyphae
Fig. 6, (Gray, 1873) passion_flower_tendrils.jpg

Handout:
Fig. 42.1 (Purves etal., 1998) sensors.jpg
Fig. 7.9 (Eckert and Randall, 1978) velocity_detector.jpg
Fig. 7.10 (Eckert and Randall, 1978) tonic_phasic.jpg

References:

Alberts B., D. Bray, J. Lewis, M. Raff, K. Roberts, J.D. Watson- 1994-<u>Molecular Biology of the Cell</u>, 3rd edition. Fig. 4.23. Garland Publishing Inc. N.Y., N.Y.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Sensory and Motor Mechanisms. Chapter 50. Pages 1087-1092, 1094-1096, 1099-1100. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Fig. 49.8. Benjamin Cummings Press. San Francisco, CA.

Corcoran A.J., J.R. Barber, W.E. Conner- 2009-Tiger moth jams bat sonar- Science 325: (#5938, 7/17) 325-327

Eckert R., D. Randall- 1978-<u>Animal Physiology</u>. Figures 7.9, 7.10, 7.38. Pgs. 216-218. W.H. Freeman and Co. San Francisco, CA.

Gould J.L., W.T. Keeton- 1996-<u>Biological Sciences</u>, 6th edition. Fig. 38.38. W.W. Norton & Company. N.Y., N.Y.

Gray A- 1873-<u>Botany for Young People. Part II. How Plants behave: How they move, climb, employ insects to work for them</u>. Fig. 6. Ivison, Blakeman, Taylor and Co., N.Y., N.Y.

Hemmersbach R., E. Becker, W. Stockem- 1997-Influence of extremely low frequently electromagnetic fields on the swimming behavior of ciliates- Bioelectromagnetics 18: 491-498

Hoch H- 2002-Contact sensing: A tactilely important phenomenon for fungi- Plant Biology Seminar, Cornell University. 11/15/02, 404 Plant Science Building.

Purves W.K., G.H. Orians, H.C. Heller, D. Sadava- 1998-<u>Life: The Science of Biology</u>. 5th edition. Fig. 42.1. Sinauer Associates Inc. Sunderland, MA.

Walcott C- 2003-Lecture #30, Neuroethology- BIO 102 Lecture notes, Fall 2003. 12/1/03, Cornell University. Ithaca, N.Y.

Wayne R., M.P. Staves- 1996-A down to earth model of gravisensing or Newton's Law of Gravitation from the apple's perspective- Physiologia Plantarum 98: 917-921

Weiss M.R- 1995-Floral color change: A widespread functional convergence- American Journal of Botany 82: (#2) 167-185

Related issues:

What happens when the sense organs are **misswired** to the brain? One result is a syndrome called **Synesthesia**. Another can result when **itch** receptors are over stimulated.

> Miller G- 2007-Grasping the clues to the biology of itch- Science 318: (#5848, 10/12) 188-189
>
> Ramachandran V.S., E.M. Hubbard- 2003-Hearing colors, tasting shapes- Scientific American 288: (#5) 52-59

For more general information on senses and aspects of them in **animals**:

> Axel R- 1995-The molecular logic of smell- Scientific American 273: (#4) 154-159
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> Catania K- 2010-Worm charmers- Scientific American 302: (#3, March) 72-76
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> Chandrashekar J., D. Yamolinsky, L. von Buchholtz, Y. Oka, W. Sly, N.J.P. Ryba, C.S. Zuker- 2009-The taste of carbonation- Science 326: (#5951, 10/16) 443-445
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> Hildebrand M., G. Goslow- 2001-Sense organs. Chapter 19, pgs 345-367, in Analysis of Vertebrate Structure. 5th edition. John Wiley & Sons, Inc. N.Y., N.Y.
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> Hu J., C. Zhang, C. Ding, Q. Chi, A. Waiz, P. Mombaerts, H. Matsunami, M. Luo- 2007-Detection of near-atmospheric concentration of CO_2 by an olfactory subsystem in the mouse- Science 317: (#5840, 8/17) 953-957
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> Huang L., A. Treisman, H. Pashler- 2007-Characterizing the limits of human visual awareness- Science 317: (#5839, 8/10) 823-825
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> Miller G- 2009-Enzyme lets you enjoy the bubbly- Science 326: (#5951, 10/16) 349
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> Shepherd G.M- 1988-The somatic senses- Chapter 12, pgs. 247-269, in Neurobiology, 2nd edition. Oxford University Press, N.Y.
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> Shepherd G.M- 1988-Muscle sense and kinesthesia- Chapter 13, pgs. 270-285, in Neurobiology, 2nd edition. Oxford University Press, N.Y.
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Senses in a **protist**:

> Jacobs W.P- 1994-*Caulerpa*- Scientific American (Dec.) 100-105

Ultraviolet (UV) perception in some organisms:

> Lim M.L.M., M.F. Land, D. Li- 2007-Sex-specific UV and fluorescence signals in jumping spiders- Science 315: (1/26) 481

Sensing in **bacteria**:

Swartz T.E., T-S. Tseng, M.A. Frederickson, G. Paris, D.J. Comerci, G. Rajashekera, J-G. Kim, M.B. Mudgett, E.A. Splitter, R.A. Ugalde, F.A. Goldbaum, W.R. Briggs, R.A. Bogomolni- 2007-Blue-light-activity histidine kinases: Two-component sensors in bacteria- Science 317: (#5841, 8/24) 1090-1093

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Senses in **fungi**:

Griffin D.H- 1994-The physical environment and growth- Chapter 7, pgs. 195-214, in Fungal Physiology- 2nd edition. Wiley-Liss Press, N.Y.

Lipan O- 2008-Enlightening rhythms- Science 319: (#5862, 1/25) 417-418

Electromagnetic field sensing in animals:

Bohannon J- 2007-Seeking nature's inner compass- Science 318: (#5852, 11/9) 904-907

Fields R.D- 2007-The shark's electric sense- Scientific American 297: (#2, Aug.) 74-81

This article describes how amino acids in a domain of a protein can function in the sensing of **electrical fields**.

Tao X., A. Lee, W. Limapichat, D.A. Dougherty, R. MacKinnon- 2010-A gating charge transfer carrier in voltage sensors- Science 328: (#5974, 4/2) 67-73

Here is a study examining the development of the **lateral line system** of fish used to detect pressure gradients in the water.

Nechiporuk A., D.W. Raible- 2008-FGF-dependent mechanosensory organ patterning in zebrafish- Science 320: (#5884, 6/27) 1774-1777

On the sense of **touch**:

Catania K.C- 2002-The nose takes a starring role- Scientific American 287: (#1) 54-59

Miller G- 2009-Fingerprints enhance the sense of touch- Science 323: (#5914, 1/30) 572-573

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Concerning some senses in **plants**:

Cahill J.F. jr, J.P. Castelli, B.B. Casper- 2002-Separate effects of human visitation and touch on plant growth and herbivory in an old-field community- American Journal of Botany 89: (#9) 1401-1409

Hamant O., M.G. Heisler, H. Jönsson, P. Krupinski, M. Uyttewaal, P. Bokov, F. Corson, P. Sahlia, A. Boudaoud, E.M. Meyerowitz, Y. Couder, J. Traas- 2008-Developmental patterning by mechanical signals in *Arabidopsis*- Science 322: (#5908, 12/12) 1650-1655

Johannes E., D.A. Collings, J.C. Rink, N.S. Allen- 2001-Cytoplasmic pH dynamics in maize pulvinal cells induced by gravity vector changes- Plant Physiology 127: 119-130

Telewski F.W- 2006-A unified hypothesis of mechanoperception in plants- American Journal of Botany 93: (#10) 1466-1476

This article describes a system for detecting tension, a **mechanosensory system**.

Paszek M., V. Weaver- 2010-Enforcing order on signaling- Science 327: (#5971, 3/12) 1335-1336

Salaita K., P.M. Nair, R.S. Petit, R.M. Neve, D. Das, J.W. Gray, J.T. Graves- 2010-Restriction of receptor movement alters cellular response: Physical force sensing by EphA2- Science 327: (#5971, 3/12) 1380-1385

BIO 107   2010
Day 13, Lecture 32, Title: Comparative Physiology.
Text Readings: Campbell et al. (2008), pgs. 708-723, 862-872, 881, 891-893, 900-903, 917-918, 920-921, 925-927, 954-960, 968-969, 1000-1001.

Topics to cover:
        Groups and Traits we will consider
        Fish
        Frogs
        Reptiles and Birds
        Mammals

Groups and Traits we will consider
        Fig. 40.2, swimming collage

        Often see various animals able to carry out similar functions.
                In contrast, differences in traits allow different abilities
                        and survival in differing habitats.
                Will consider some variations in a few traits seen in a few vertebrate groups.
                Notice that no set of traits is "best" in all cases... depends on habitat...

        Groups: Fish, Frogs, Reptiles (including Birds), Mammals
        Traits include: Thermoregulation, Osmoregulation, Digestive Systems,
                Gas Exchange and Circulation, and Reproductive Systems.

Fish
        Fig. 34.17, Ray finned fish diversity
        Habitats include fresh and marine waters.
                Differ in temperatures, osmolarity, food sources, etc....
                So fishes are diverse in their traits...
        Thermoregulation
                Ectothermy versus endothermy
                Fig. 40.7, Endo Ecto Thermoregulation
                        Endotherms tend to hold their internal temperatures stable.
                        Most fish are ectotherms, so internal temperatures vary with environment
                              but in the ocean the environment does not vary rapidly...
                        Consider range of variation of marine water temperatures...
                              not likely to encounter sub-zero temperatures...
                Fig. 40.16, (Campbell and Reece, 2005) Aquatic Thermoregulation
                        Consider bluefin tuna and great white shark
                              use counter-current blood flow to hold heat in body core
                              keeps body core warmer than periphery

This may be adaptive for active predators, especially in cold waters?
but costs metabolic energy...

Gas Exchange and Circulation
Fig. 42.22, Fish Gills
See Counter current exchange in gills, to pull out oxygen well
Since fish are mostly ectothermic, internal temperature and metabolism changes
this makes their oxygen demand low, so why this system in the gills?
Figure 45_2 Water Breathers.jpg (Purves et al., 1998)
But water holds less oxygen when warm
So a limit of activity of fish in warmer waters
(Implications of this as oceans warm with climate change?)
Once have oxygen, low demand allows use of a two chambered heart
Fig. 42.4, Fish Circulation
ectothermy gives low oxygen demand
buoyancy in water gives low oxygen demand
so once has oxygen can survive with two capillaries in serries
if water oxygen content is good enough
So limited in habitat to cooler waters and aerobic waters...

Osmoregulation
Many fish keep their body osmolarity distinct from the environment.
Fig. 44.4, Osmoregulation
Marine fish lose water to sea
must create concentrated urine and recover water well
often use urea as nitrogenous waste
Fresh water fish take in lots of water from lake
must create dilute water, and recover salts very well
often use ammonia as nitrogenous waste
Others make their internal osmolarity similar to that of the enviroment
Figure 53_6 Great White Shark.jpg (Raven and Johnson, 1995)
sharks do this by using urea as a osmotica, as not toxic

Digestive System
Characteristics of it can tell you the food type consumed
Fig. 34.16, Trout Anatomy
Figure 27_29 Digestive tract of shark.jpg (Gould and Keeton, 1996)
Both are short digestive tracts,
implies eats easily digested and absorbed food
generally that means carnivorous
Figure 12_6 digestive tracts of an Elasmogranch and a Teleos.jpg
(Hildebrand and Goslow, 2001)
Note spiral valve of shark, aids surface area of intestinal tract
Note Pyloric caeca in trout, also adds surface area...

Reproduction
        In water so gametes do not dry out, so external fertilization is common
        Gametes can be viewed by other speceis as "fish food", so release many of them
        May have some behavioral timing of gamete release

Frogs
    Fig. 34.21, amphibians
    Thermoregulation
        Ectothermic, so low metabolic cost.
        adult can go dormant at bottom of pond, a benefit for overwintering
    Osmoregulation
        Excrete dilute urine, as live in freshwater, often use ammonia as N-waste.
        skin does not resist water loss...
    Digestive System
        Figure 27_24 Intestines adult frog and tadpoles.jpg (Gould and Keeton, 1996)
        As adult is a carnivore, as a tadpole is a herbivore
        What advantage to this distinct set of foods at different stages of life?
            what does this do to the number of tadpoles and adult frogs in an area?
    Gas Exchange and Circulation
        Tadpole uses gills and is water breathing.
        Adult is air breathing and breaths through skin
        So again, another major shift in physiological system
            Fig. 42.5, Vertebrate Circulation
            (recall goof in this figure,
                lungs and skin do not share the same capillary bed)
            so must get oxygenated blood either from skin, if underwater,
                or from lungs, if has access to air...
    Reproduction
        Fig. 34.22, Frog Life Stages
        See again many gametes released,
        Can time gamete release for when growth conditions are good.
    So frogs are adaptive to wet habitats, at least seasonal ponds...
        due to:
            external fertilization, tadpole stage, skin breathing...

Reptiles and Birds
    Fig. 34.30, Bird Diversity
    Fig. 34.27, reptile diversity
        Recall that birds are one type of reptile...
    Thermoregulation
        Most Lizards, turtles, crocodiles, etc...   are ectothermic
        But Birds are endothermic, as need active muscles for flight...
        So see a big difference here.

Lizards can use behaviors to modify their temperature
> Fig. 40.9, Lizard on Rock
> Figure 37_14, lizard behavioral theromregulation.jpg (Purves et al., 1998)
> Can be warm during day, if day is sunny and warm.

Being ectothermic can save lizards a great deal of energy
> Fig. 40.10, (Campbell and Reece, 2005) Energy Budgets
> Fig. 40.20, energy budgets
> so can survive on less food per year
>> use less energy to maintain unit of body mass
> Good adaptation for living in area of low primary productivity...

Birds are endotherms
> use feathers to keep in heat, note also: hollow bones for less mass
>> Fig. 34.28, Wing Structure
> Can use counter current systems to have cold feet in cold water
>> Fig. 40.12, Counter Current Thermoregulation
>> saving heat, saves metabolic costs

Consequences of amniotic egg
> Fig. 34.25, Amniote Egg
> early stages of growth are ectothermic
> so warm mother bird sit on cool eggs

Osmoregulation
> these are amniotes, so lay amniotic eggs, with either leathery or mineral covering
> with amniotic egg no more need for standing freshwater
> but egg may still risk drying out, so uses uric acid as waste to conserve water
>> use of uric acid as N-waste retained in adult as well
>> so here the adult nitrogenous waste is essential when in the egg,
>>> not essential as an adult
> If eat salty food, or drink sea water, can use salt gland
>> Fig. 44.7, Bird Salt Gland
>> Fig. 44.8, Bird Salt Gland
>> This allows eatting of marine animals,
>> high in protein and oils, good as a food source for an endotherm like a bird

Digestive System
> There are some vegetarian birds, can see this in their digestive tracts...
>> Figure 27_31 Hoatzin vegetative bird.jpg (Gould and Keeton, 1996)
> Figure 12_7 Digestive tracts of Reptile and Granivorous Bird.jpg
>> (Hildebrand and Goslow, 2001)
> Birds lack of teeth
>> implies need for gizzard...can shread up food, but gizzard grinds it up.
>> any reptile that has teeth will not have a gizzard...

Gas Exchange and Circulation
    Birds have one-way air flow through lungs, improves oxygen extraction
        Fig. 42.26, Bird Respiratory System
    Birds, need good oxygen supply, as have high metabolic demand due to flight
        and are endotherms
    Some reptiles do gas exchange through their skin as well as in lungs
        Fig. 42.5, Vertebrate Circulation
        so need a pulmocutaneous circuit like frogs...
        turtles exchange gas in cloaca, crocodiles through roof of mouth
        need four chambered heart and shunts...
    Note that some reptiles have a shunt between two circulatory loops that
        seem similar to mammalian fetal circulation
        Figure 46_4 Vertebrate circulatory systems.jpg (Purves et al., 1998)
            recall the *ductus arteriosus* of fetal circulation?
            recall the *foramen ovale*?
Reproduction
    Use internal fertilization, so gametes do not dry out
        this is consistent with amniotic egg resisting dessication...
    So may have sexual dimorphism, produce fewer gametes...
        Fig. 51.23, Sexual Dimorphism
    And can have quality care for eggs that are laid
    How to hatch out of egg
        Fig. 34.26, Hatching Reptile
        Some birds have special bone to crack shell
            Figure 5_1 shellbreaker structure in birds.jpg
                (Hildebrand and Goslow, 2001)
So many of reptile adaptations good for living on land
    bird also has good adaptations for flight.

Mammals
    Thermoregulation
        Have previously discussed hormonal controls of metabolic rates
            note role of fur, and fatty layers in skin...
        There are also behavioral options...
            Fig. 44.07, (Campbell and Reece, 2002) Hippo Thermoregulation
            Fig. 40.18, (Campbell and Reece, 2005) Elephant Thermoregulation
        Generally larger sized body associated with lower cost per mass
            Fig. 40.10, (Campbell and Reece, 2005) Energy Budgets
            Fig. 40.19, energy cost per body mass
            So if losing heat, tend to see selection for larger size body
                think of whales in the arctic ocean...

Osmoregulation
  Kangaroo rat, has long nephrons, so produces very concentrated urine
    Fig. 44.6, Water Balance Comparison
    Fig. 44.16b, Kangaroo Rat (Campbell and Reece, 2002)
    So does not need to drink, and can get water from cellular respiration
Digestive System
  See same carnivore versus herbivore changes in digestive tract
    Fig. 41.19, Digestive tract comparison
    Can also have rumen and do fermentative digestion...
  Can also tell pattern of eatting from skull and teeth pattern in bones
    Fig. 41.18, dentition and diet
    note canine teeth versus molar teeth
Gas Exchange and Circulation
  Changes in Llama hemoglobin allows better high altitude living
    Figure 45_15 Llama hemoglobin.jpg (Purves et al., 1998)
    Note similar change as seen in human fetal hemoglobin?
  Early human embryo
    Fig. 47.16, Human Early Embryo
    when small uses diffusion
    later grows placenta and circulatory system
    This allows it to do gas exchange through a non-lung capillary bed
      Figure 55_19 Human fetal circulation.jpg
        (Raven and Johnson, 1995)
      Somewhat similar to what is done by some reptiles?
    Figure 46_4 Vertebrate circulatory systems.jpg (Purves et al., 1998)
Reproduction
  Use of internal fertilization, and complex pre-zygotic behaviors
  Figure 39_8 Rhino Internal Fertilization.jpg (Purves et al., 1998)

So no one set of traits is best for all habitats.
Change the habitat and a new set of traits may be more adaptive for it.
Each animal species has its set of habitats in which it can live.
  Some are narrow, some broad.
  The combination of physiological traits present in the animal does relate to its success
    in its habitats.

Objectives:

Be able to describe how the traits present in the groups of animals covered (fish, frogs birds and other reptiles, and mammals) helps makes each of the animals adaptive for the typical habitat in which each is found.

What effect can endothermy or ectothermy have on the demands for gas exchange and on the circulatory system found in a species? What are the costs and benefits of ectothermy compared to endothermy, and what types of habitats would you expect to find each? How can counter-current exchange systems be used to retain heat, and what other adaptations for heat retention are used by some vertebrates?

What adaptations are seen in some vertebrate groups that makes them more adaptive to different feeding patterns, such as carnivory versus herbivory?

In what ways are the circulatory systems found in frogs and in some reptiles adaptive for doing gas exchange through various systems in their bodies? Fish have relatively simple circulatory systems, how is this possible?

Be able to describe several adaptations found in land dwellers, and others found in marine species, that address the need to retain water.

What are the costs and benefits of external compared to internal fertilization? What adaptations tend to be associated with each?

Not just the adults, but also earlier stages in the life cycle must be adaptive. Describe traits found in each of the following and how they make them more adaptive: tadpoles, chicks in the egg, and mammalian fetuses.

Needed overheads and items:

Fig. 40.2, swimming collage
Fig. 34.17, Ray finned fish diversity
Fig. 40.7, Endo Ecto Thermoregulation
Fig. 40.16, (Campbell and Reece, 2005) Aquatic Thermoregulation
Fig. 42.22, Fish Gills
Figure 45_2 Water Breathers.jpg (Purves et al., 1998)
Fig. 42.4, Fish Circulation
Fig. 44.4, Osmoregulation
Figure 53_6 Great White Shark.jpg (Raven and Johnson, 1995)
Fig. 34.16, Trout Anatomy
Figure 27_29 Digestive tract of shark.jpg (Gould and Keeton, 1996)
Figure 12_6 digestive tracts of an Elasmogranch and a Teleos.jpg (Hildebrand and Goslow, 2001)
Fig. 34.21, amphibians
Figure 27_24 Intestines adult frog and tadpoles.jpg (Gould and Keeton, 1996)
Fig. 42.5, Vertebrate Circulation
Fig. 34.22, Frog Life Stages
Fig. 34.30, Bird Diversity
Fig. 34.27, reptile diversity
Fig. 40.9, Lizard on Rock
Figure 37_14, lizard behavioral theromregulation.jpg (Purves et al., 1998)
Fig. 40.10, (Campbell and Reece, 2005) Energy Budgets
Fig. 40.20, energy budgets
Fig. 34.28, Wing Structure
Fig. 40.12, Counter Current Thermoregulation
Fig. 34.25, Amniote Egg
Fig. 44.7, Bird Salt Gland
Fig. 44.8, Bird Salt Gland
Figure 27_31 Hoatzin vegetative bird.jpg (Gould and Keeton, 1996)
Figure 12_7 Digestive tracts of Reptile and Granivorous Bird.jpg (Hildebrand and Goslow, 2001)
Fig. 42.26, Bird Respiratory System
Fig. 42.5, Vertebrate Circulation
Figure 46_4 Vertebrate circulatory systems.jpg (Purves et al., 1998)
Fig. 51.23, Sexual Dimorphism
Fig. 34.26, Hatching Reptile
Figure 5_1 shellbreaker structure in birds.jpg (Hildebrand and Goslow, 2001)
Fig. 44.07, (Campbell and Reece, 2002) Hippo Thermoregulation
Fig. 40.18, (Campbell and Reece, 2005) Elephant Thermoregulation
Fig. 40.10, (Campbell and Reece, 2005) Energy Budgets
Fig. 40.19, Energy costs per body mass
Fig. 44.6, Water Balance Comparison

Fig. 44.16b, Kangaroo Rat (Campbell and Reece, 2002)
Fig. 41.19, Digestive tract comparison
Fig. 41.18, dentition and diet
Figure 45_15 Llama hemoglobin.jpg (Purves et al., 1998)
Fig. 47.16, early human embryo
Figure 55_19 Human fetal circulation.jpg (Raven and Johnson, 1995)
Figure 46_4 Vertebrate circulatory systems.jpg (Purves et al., 1998)
Figure 39_8 Rhino Internal Fertilization.jpg (Purves et al., 1998)

Handout:
    (Handout - Lecture 32 - Comparative Physiology.stm)
    Figure 45_2 Water Breathers.jpg (Purves et al., 1998)
    Figure 37_14, lizard behavioral theromregulation.jpg (Purves et al., 1998)
    Figure 46_4 Vertebrate circulatory systems.jpg (Purves et al., 1998)
    Figure 55_19 Human fetal circulation.jpg (Raven and Johnson, 1995)

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Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Biology. Eighth edition. Pages 708-723, 862-872, 881, 891-893, 900-903, 917-918, 920-921, 925-927, 954-960, 968-969, 1000-1001. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-Biology, 7th edition, Figures 40.10, 40.16, 40.18. Benjamin Cummings Press. San Francisco, CA.

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Hildebrand M., G. Goslow- 2001-Analysis of Vertebrate Structure. 5th edition. Fig. 5.1, 12.6, 12.7. John Wiley & Sons, Inc. N.Y., N.Y.

Purves W.K., G.H. Orians, H.C. Heller, D. Sadava- 1998-Life: The Science of Biology. 5th edition. Fig. 37.14, 39.8, 45.15, 46.4. Sinauer Associates Inc. Sunderland, MA.

Raven P.H., G.B. Johnson- 1995-Biology, 3rd edition. Fig. 53.6, 55.19. Wm.C. Brown Publishers. Dubuque, Iowa.

Related issues:

For a good text of background material see:

> Hildebrand M., G. Goslow- 2001-<u>Analysis of Vertebrate Structure</u>. 5th edition. John
> Wiley & Sons, Inc. N.Y., N.Y.

Fish need to get oxygen from the water.

> Pörtner H.O., K. Knust- 2007-Climate change affects marine fishes through oxygen
> limitation of thermal tolerance- Science 315: (1/5) 95-97

Fish often have tricks to help **catch food**. Such as fluorescent lures.

> Haddock S.H.D., C.W. Dunn, P.R. Pugh, C.E. Schnitzier- 2005-Bioluminescent and red-
> fluorescent lures in a deep-sea siphonophore- Science 309: (7/8) 263

The **physiology** of many species goes through **cycles** over days or seasons.

> Busino L., F. Bassermann, A. Maiolica, C. Lee, P.M. Nolan, S.I.H. Godinho, G.F.
> Draetta, M. Pagano- 2007-SCF^{Fbx13} controls the oscillation of the circadian clock
> by directing the degradation of cryptochrome proteins- Science 316: (5/11) 900-
> 904
>
> Godinho S.L.H., E.S. Maywood, L. Shaw, V. Tucci, A.R. Barnard, L. Busino, M.
> Pagano, R. Kendall, M.M. Quwailid, M.R. Romero, J. O'Neill, J.E. Chesham, D.
> Brooker, Z. Lalanne, M.H. Hastings, P.M. Nolan- 2007-The after-hours mutant
> reveals a role for fbz13 in determining mammalian circidian period- Science 316:
> (5/11) 897-900
>
> Kang J-E., M.M. Lim, R.J. Bateman, J.J. Lee, L.P. Smyth, J.R. Cirrito, N. Jujiki, S.
> Nishino, D.M. Holltzman- 2009-Amyloid-β dynamics are regulated by orexin and
> the sleep-wake cycle- Science 326: (#5955, 11/13) 1005-1007
>
> Lamia K.A., U.M. Sachdeva, L.D. Tacchio, E.C. Williams, J.G. Alvarez, D.F. Egan, D.S.
> Vasquez, H. Juguilon, S. Panda, R.J. Shaw, C.B. Thompson, R.M. Evans- 2009-
> AMPK regulates the circadian clock by cryptochrome phosphorylation and
> degradation- Science 326: (#5951, 10/16) 437-440
>
> Lincoln G.A., I.J. Clarke, R.A. Hut, D.G. Hazlerigg- 2006-Characterizing a mammalian
> circannual pacemaker- Science 314: (12/22) 1941-1944
>
> McDearmon E.L., K.N. Patel, E.H. Ko, J.A. Walisser, A.C. Schook, J.L. Chong, L.D.
> Wilsbacher, E.J. Song, H-K. Hong, C.A. Bradfield, J.S. Takehashi- 2006-
> Dissecting the functions of the mammalian clock protein BMAL1 by tissue-
> specific rescue in mice- Science 314: (11/24) 1304-1308

One of these papers relates body **temperature regulation** in mice to their **lifespan**. The others note other factors relating to **aging** in animals.

Conti B., M. Sanchez-Alavez, R. Winsky-Sommerer, M.C. Morale, J. Lucero, S. Brownell, V. Fabre, S. Huitron-Resendiz, S. Henriksen, E.P. Zorrilla, L. deLecea, T. Bartfai- 2006-Transgenic mice with a reduced core body temperature have an increase life span- Science 314: (11/3) 825-828

Kaeberlein M., P. Kapoki- 2009-Aging is RSKy business- Science 326: (#5949, 10/2) 55-56

Selman C., J.M.A. Tullet, D. Wieser, E. Irvine, S.J. Lingard, A.I. Chondhury, M. Claret, H. Al-Qussab, D. Carmignac, F. Ramadani, A. Woods, I.C.A. Robinson, E. Schuster, R.L. Batterham, S.C. Kozma, G. Thomas, D. Carling, K. Okkenhaug, J.M. Thornton, L. Partridge, D. Gems, D.J. Withers- 2009-Ribosomal protein S6 kinase 1 signaling regulates mammalian life span- Science 326: (#5949, 10/2) 140-144

They are beginning to get an idea of how **patterns of gene expression** relates to the final **body size** that an animal species can have.

Crickmore M.A- 2009-The molecular basis of size differences- Science 326: (#5958, 12/4) 1360-1361

BIO 107   2010
Day 14, Lecture 33. Title: Animal Behavior.

Text Readings: Campbell et al. (2008), Chapter 51.

Topics to cover:

What is behavior?
Innate Behavior
  Fixed Action Patterns and Sign Stimuli
  Kinesis and Taxis
  Genetic Basis
Learnt Behavior
  Habituation
  Imprinting
  Operant and Classical Conditioning
More complex behaviors
  Ritual Behaviors
  Kin Selection
  Bee Communication

What is behavior?
  For now, follow the description given in the text...
  (Campbell et al., 2008; pg. 1120)
"An individual behavior is an action carried out by muscles or glands under control of the nervous system in response to a stimulus."

What then is behavior?
  An emergent property of life.
  How does behavior differ from growth?
    repeatable vs. permanent changes
  Uses signal transduction systems.
        both influenced by outside stimuli
        both involves signal reception, integration, and response
  May be learnt or innate.

  If subjected to selection, may be adaptive
    result in more survival to reproduce
    individuals with adaptive behaviors may mate more
        and pass on their genetics more

1

Innate Behavior

These are genetically based and so if grow normally will show up

Thus do not need to be learnt...

Though may depend on maturation of certain structures to operate, i.e. muscles...

Fixed Action Patterns and Sign Stimuli

Fixed action patterns

Implies fixed neuronal connections, genetically determined

Fig. 35.38 (Gould and Keeton, 1996) Aplysia_feeding.jpg

sterotypical, once started tends to continue to completion

ex. bird nest building

Sign stimuli

Fig. 51.3, stickle back fish behavior

note male red belly acts to elicit a response from female

gets a different response from male

environmental signals response is targeted on, super sign stimuli

Kinesis and Taxis

These are two of many patterns of innate behavior

Kinesis, one type of innate behavior

Fig. 51.4, kinesis

a change in rate of movement or turning

consider an animal in cool area, best if stays there

so either moves more slowly or turns more to stay there

consider animal in hot area

goes faster or in straight line to get away

Taxis, another type of innate behavior

Fig. 51.7b, (Campbell and Reece, 2005) taxis

change in direction or orientation relative to direction of source of stimulus

unlike kinesis this is relative to stimulus

ex: fish and stream flow, phototaxis by insects

Positive taxis, towards stimulus

Negative taxis, away from stimulus

ex: Positive or negative phototaxis...

Genetic Basis

Evidence of genetic basis of behavior.

If based on genes, then mutations in genes should modify behavior.

Here is one example: Lovebird nest building example

Fig. 51.1, (Campbell and Reece, 2002) bird tucking behavior

One strain carries nest material in beak, other tucks in tail

note breeding to form hybrid

get behavior which is intermediate

like inheriting a fixed action pattern in part?

Note, if not at correct developmental state may not show behavior

so need to look at behavior at proper developmental stage...

Another example: insect songs.

If genetically based then breeding should alter it...

Fig. 51.14, insect song genetics

Some evidence that SOME insect species have a genetic basis for their song

hybrid has intermediate features: amplitude of one, period of other

Learnt Behavior

The capacity to learn may be genetically based,

There may be in a species a better capacity to learn certain items than others.

Can change capacity over time, i.e. language learning capacity...

But learning requires exposure to stimuli, practice, etc...

Habituation

One type of learning

Fig. 48.31, (Campbell and Reece, 2005) Aplysia learning

Fig. 41.18, (Purves etal., 1998) Aplysia_touch.jpg

repeated touch can desensitize, and so it no longer responds

an applied shock can sensitize again...

Can relate this learning to states of neurons and synapses

Imprinting

another form of learning, occurs during a sensitive period

Fig. 51.5, (Campbell and Reece, 2005) geese imprinting

example of chicks imprinting on mom

adaptive as keeps chicks near parent

Konrad Lorenz and chicks

Fig. 51.10, Lorenz imprinting

Note many birds only learn songs during a sensitive period,

Or may be able to produce songs only during certain times of year

something must change in brain?

Fig. 51.10, (Campbell and Reece, 2002) bird song learning

Operant and Classical Conditioning
Operant conditioning
  trial and error learning
  Example of coyote with face of quills
  Fig. 51.12, operant conditioning

Classical conditioning
  association of stimulus and reward
  such as cookies and exam??
  Fig. 51.11, wasp nest-location
  use of markers to locate nest
  learning of visual cues, association of them with nest

More complex behaviors.
 these involve complex combinations of other behaviors.
 are not easily cataloged into one type

Ritual Behaviors
Ritual behavior is also called agonistic behavior
Fig. 51.19, (Campbell and Reece, 2002) snake ritual wrestling
  advantages to actual fighting...
  seen in bears, wolves, etc...
Courtship behavior
Fig. 51.23, (Campbell and Reece, 2002) stickleback fish mating
a complex mix of stimuli and responses

Kin Selection
Concept of altruism and kin selection
Fig. 51.28, genetics of sibling relationship
  siblings have r of 0.5
  Aunt and niece, r = 0.25
  first cousins, r = 0.125
  So can estimate relatedness, and see if this influences behaviors..
Fig. 51.29, kin selection and altruism
  females nearer to burrow
  put self at risk to warn close relatives
  a type of altruistic behavior

Bee Communication

Communication as a complex behavior. An example is Bee language

Fig. 51.8, bee communication

        note angle relative to sun

        duration of dance proportional to distance

        Different dance for close items

        different patterns by hive/species

So behavior is an emergent property

        can be innate (i.e. genetic), or learnt (i.e. not directly coded in genes)

        involves stimulus, integration, response

                as we've seen before

        From these examples, think about what is needed for behavior.

                What about at cellular, tissue, organ, organismal levels....?

Objectives:

How does a behavioral response compare with a growth response? What is similar and what is different? We call behavior an emergent property, but from what does it emerge? What components are needed in animals in order to have a behavior?

What must be seen in a behavior for it to be taken as evidence of habituation? What is needed to show a taxis? A kinesis? A fixed action pattern? Be able to describe these examples in terms of likely signal reception, transduction, and response processes at the cellular, tissue, and organismal levels.

How would you determine whether a behavior is innate or is learnt? Be able to describe examples of each type of behavior. How does operant conditioning differ from classical conditioning? Be able to describe examples of each that might be observed in the wild. What is imprinting and is it purely innate or learnt behavior? Is the capacity for, or use of, language an innate or a learnt behavior?

Is altruistic behavior adaptive for an individual or for a species or for both or neither? How so? Relate this to specific examples of altruistic behaviors. Be able to calculate relative genetic relatedness and describe the arguments that relate this to how altruistic behaviors may be adaptive.

For review, see self-quiz questions #1, 3, and 6 at the end of this chapter.

Needed overheads and items:

Fig. 35.38 (Gould and Keeton, 1996) Aplysia_feeding.jpg
Fig. 51.3, stickleback models
Fig. 51.4, kinesis
Fig. 51.7b, (Campbell and Reece, 2005) taxis
Fig. 51.1, bird tucking behavior (Campbell and Reece, 2002)
Fig. 51.14, insect song genetics
Fig. 48.31, (Campbell and Reece, 2005) Aplysia learning
Fig. 41.18, (Purves etal., 1998) Aplysia_touch.jpg
Fig. 51.5, (Campbell and Reece, 2005) geese imprinting
Fig. 51.10, Lorenz imprinting
Fig. 51.10, bird song learning (Campbell and Reece, 2002)
Fig. 51.12, operant conditioning
Fig. 51.11, Wasp nest location
Fig. 51.19, snake ritual wrestling (Campbell and Reece, 2002)
Fig. 51.23, stickleback fish mating (Campbell and Reece, 2002)
Fig. 51.28, genetic relatedness between siblings
Fig. 51.29, kin selection and altruism
Fig. 51.8, bee communication

Handout:
Lecture 33-Animal Behavior - Handout.stm
Fig. 35.38 (Gould and Keeton, 1996) Aplysia_feeding.jpg
Fig. 51.1, bird tucking behavior (Campbell and Reece, 2002)
Fig. 41.18, (Purves etal., 1998) Aplysia_touch.jpg
(Slide of calculation of relatedness...)

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Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Animal behavior. Chapter 51. Pages 1120-1145. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

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Gould J.L., W.T. Keeton- 1996-<u>Biological Science</u>. 6th edition. Fig;. 35.38. W.W. Norton & Co. N.Y., N.Y.

Purves W.K., G.H. Orians, H.C. Heller, D. Sadava- 1998-<u>Life: The Science of Biology</u>. 5th edition. Fig. 41.18. Sinauer Associates Inc. Sunderland, MA.

Related issues:

For more on **animal behavior** seen in various species see:

Antsey M.L., S.M. Rogers, S.R. Ott, M. Burrows, S.J. Simpson- 2009-Serotonin mediates behavioral gregarization underlying swarm formation in desert locusts- Science 323: (#5914, 1/30) 627-630

Barbero F., J.A. Thomas, S. Bonelli, E. Balletto, K. Schönrogge- 2009-Queen ants make distinctive sounds that are mimicked by a butterfly social parasite- Science 323: (#5915, 2/6) 782-785

Cator L.J., B.J. Arthur, L.C. Harrington, R.R. Hoy- 2009-Harmonic convergence in the love songs of the dengue vector mosquito- Science 323: (#5917, 2/20) 1077-1079

Chapman J.W., R.L. Nesbit, L.E. Burgin, D.R. Reynolds, A.D. Smith, D.R. Middleton, J.R. Hill- 2010-Flight orientation behaviors promote optimal migration trajectories in high-flying insects- Science 327: (#5966, 2/5) 682-685

Cohen J- 2007-The world through a chimp's eye- Science 316: (4/6) 44-45

Dickson B.J- 2008-Wired for sex: The neurobiology of *Drosophila* mating decisions- Science 322: (#5903, 11/7) 904-909

Galicia C.G- 2007-Brainwashing, honey bee style- Science 317: (#5836, 7/20) 326-327

Gibbons A- 2007-Spear-wielding chimps seen hunting bush babies- Science 315: (2/23) 1063

Heinrich B., T. Bugnyar- 2007-Just how smart are ravens?- Scientific American 296: (#4, April) 64-71

Maggioncalda A.N., R.M. Sapolsky- 2002-Disturbing behaviors of the orangutan- Scientific American 286: (#6) 60-65

Mealey L- 2000-Male strategies and tactics. Chapter 5, pgs. 87-115. In, <u>Sex differences. Developmental and Evolutionary Strategies</u>. Academic Press, San Diego

Mealey L- 2000-Female strategies and tactics. Chapter 6, pgs. 117-142. In, <u>Sex Differences. Developmental and Evolutionary Strategies</u>. Academic Press, San Diego

Mealey L- 2000-Mating systems. Chapter 7, pgs. 143-172. In, <u>Sex Differences. Developmental and Evolutionary Strategies</u>. Academic Press, San Diego

Russell A.F., N.E. Longmore, A. Cockburn, L.B. Astheimer, R.M. Kilner- 2007-Reduced egg investment can conceal helper effects in cooperatively breeding birds- Science 317: (#5840, 8/17) 941-944

Rutz C., L.A. Bluff, A.A.S. Weir, A. Kacelnik- 2007-Video cameras on wild birds- Science 318: (#5851, 11/2) 765

Sanes D.H., T.A. Reh, W.A. Harris- 2006-Behavioral development- Chap 10, pgs 289-321, in <u>Development of the Nervous System</u>, 2nd edition. Elsevier Academic Press, N.Y., N.Y.

Travis J- 2007-Do wandering albatrosses care about math?- Science 318: (#5851, 11/2) 742-743

Here is another interesting article about efforts to make use of electronic chips embedded in human brains to **alter human behavior** ("Robocop" anyone?):

Horgan J- 2005-The forgotten era of brainchips- Scientific American 293: (#4, Oct.) 66-73

Here are studies of behaviors in humans. Our **social cognition**, **mating** behaviors, and the effects of **kinship** on humans' fertility. Also a study of the origin of human **language** abilities.

Balter M- 2010-Animal communication helps reveal roots of language- Science 328: (#5981, 5/21) 969-971

Boyd R- 2006-The puzzle of human society- Science 314: (12/8) 1555-1556

Helgason A., S. Pálsson, D.F. Guðbjartsson, P. Kristjánsson, K. Stefánsson- 2008-An association between the kinship and fertility of human couples- Science 319: (#5864, 2/8) 813-816

Herrmann E., J. Call, M.V. Hernández-Lioreda, B Hare, M. Tomasello- 2007-Humans have evolved specialized skills of social cognition: The cultural intelligence hypothesis- Science 317: (#5943, 9/7) 1360-1366

Mealey L- 2000-The human animal. Chapter 8, pgs. 175-211. In, Sex Differences. Developmental and Evolutionary Strategies. Academic Press, San Diego

Mealey L- 2000-Women's strategies and tactics. Chapter 9, pgs. 213-260. In, Sex Differences. Developmental and Evolutionary Strategies. Academic Press, San Diego

Mealey L- 2000-Men's strategies and tactics. Chapter 10, pgs. 261-304. In, Sex Differences. Developmental and Evolutionary Strategies. Academic Press, San Diego

Roberts R.B., J.R. Ser, T.D. Kocher- 2009-Sexual conflict resolved by invasion of a novel sex determiner in Lake Malawi cichlid fishes- Science 326: (#5955, 11/13) 998-1001

Ule A., A. Schram, A. Riedl, T.N. Cason- 2009-Indirect punishment and generosity toward strangers- Science 326: (#5960, 12/18) 1701-1704

These articles deal with various aspects of **social behavior** in other animals.

Balter M- 2008-Why we're different: Probing the gap between apes and humans- Science 319: (#5862, 1/25) 404-405

Boyd R., S. Mathew- 2007-A narrow road to cooperation- Science 316: (#5833, 6/29) 1858-1859

Eatherley D- 2008-Relative distance- Scientific American 298: (#1, Jan) 29-30

Hinde C.A., R.A. Johnstone, R.M. Kilner- 2010-Parent-offspring conflict and coadaptation- Science 327: (#5971, 3/12) 1371-1376

Jensen K., J. Call, M. Tomasello- 2007-Chimpanzees are rational maximizers in an ultimate game- Science 318: (#5847, 10/5) 107-109

Putterman L- 2010-Cooperation and punishment- Science 328: (#5978, 4/30) 578-579

Raihani N.J., A.S. Grutter, R. Bshary- 2010-Punishers benefit from third-party punishment in fish- Science 327: (#5962, 1/8) 171

Silk J.B- 2007-Social components of fitness in prmate groups- Science 317: (#5843, 9/7) 1347-1351

Toth A.L., K. Varala, T.C. Newman, F.E. Miguez, S.K. Hutchison, D.A. Willoughby, J.F. Simons, M. Egholm, J.H. Hunt, M.E. Hudson, G.E. Robinson- 2007-Wasp gene expression supports an evolutionary link between maternal behavior and eusociality- Science 318: (#5849, 10/19) 441-444

Van Schaik G- 2006-Why are some animals so smart?- Scientific American 294: (#4, Apr) 64-71

Vergoz V., H.A. Schreurs, A.R. Mercer- 2007-Queen pheromone blocks aversive learning in young worker bees- Science 317: (#5836, 7/20) 384-386

West S.A., A. Gardner- 2010-Altruism, spite, and greenbeards- Science 327: (#5971, 3/12) 1341-1344

Whitfield J- 2007-Who's the queen? Ask the genes- Science 318: (#5852, 11/9) 910-911

Thes articles review past studies of **primate behaviors** and **language** use. And one refers to an article in which bonobos were co-authors!

Cohen J- 2010-The inner workings of the chimpanzee brain- Science 328: (#5974, 4/2) 40-41

Cohen J- 2010-Boxed about the ears, ape language research field is still standing- Science 328: (#5974, 4/2) 38-39

Cohen J- 2010-Talking chimp to chimp- Science 328: (#5974, 4/2) 36-37

Cohen J- 2010-In the shadow of Jane Goodall- Science 328: (#5974, 4/2) 30-35

BIO 107   2010
Day 14, Lecture 34, Title: Non-Animal Behaviors.
Text Readings: Campbell et al. (2008), pgs. 207, 777-778, 821-824, 838, 842-843.

Topics to cover:

What is Behavior, and Disclaimer
Bacterial Behaviors
Protist Behaviors
Plant Behaviors
Why are Non-animal Behaviors ignored?

What is Behavior, and Disclaimer
Definition of behavior,   (Campbell et al., 2008; pg. 1120)
"An individual behavior is an action carried out by muscles or glands under control of the nervous system in response to a stimulus."
The emphasis in the text may imply that behavior is specific to just animals...?
My view: (And so contested by some others... So be warned!)
The above is too vague. Does not exclude growth?
Is behavior limited to just animals? I think not...
Clearly animals have complex behaviors,
and as animals ourselves we appreciate this.
But my view is behavior is an emergent property of LIFE, not just of animals.

I see behavior as a non-growth response to a stimulus. It is often repeatable,
and generally reversible. It is subject to selection, and so is often adaptive.
It generally involves signal reception, integration, response; therefore it should
have underlying mechanisms that can be identified and studied....

If my view is correct I should be able to find examples of behavior from even simple forms of life.
would not expect brains, nervous tissue, muscles...
would expect mostly innate behaviors, rather than ones based on learning...
Animals have complex behaviors, expect other lifeforms will have simpler behaviors.

Behavior depends, in all cases, on
signal reception, signal transduction, and response.
The key here is that the responses are NON-growth, and repeatable.
this does not mean that there is no dependence on metabolism or protein synthesis
but it does not directly involve new cells or the growth of new structures.

1

Bacterial Behaviors

      Myxococcus xanthus   nice review, bacterial kinesis (Velicer and Stredwick, 2002)

            a rod bacterium

            moves by gliding, pili pull on substrate to move, hunts in packs, surrounds prey

                  feeds on bacteria and eukaryotes

            whether move in groups, or live as individuals depends on environment

                  so is reversible

            Fig. 11.3, *Myxococcus xanthus*

                  (Note: this is from a chapter on cell communication, not behavior!)

      Reports of chemotaxis in bacteria (Adler, 1969)

            this makes sense since bacteria must sense environment, and respond by moving

      In contrast, some growth examples: involves changes in gene exp...

            Fig. 27.9, Anthrax spores

            Fig. 27.5, bacterial pili

            Clearly these are growth and differentiation that are not behavioral...

Protist Behaviors

Recall from lecture on senses:

      Magnetic sensors (Hemmersbach etal., 1997)

            Paramecium biaurelia, *Loxodes striatus*, *Tetrahymena thermophila*

            These cells exposed to fields

                  at 50 Hz, of 0.5-2.0 mT magnetic field.

                  In response see a dose dependent increase

                      in swimming velocity,

                      and a rise in the amount of turning done.

      Phototaxis

            Fig. 28.7, Euglena

            Euglena sp. swims towards the light. A phototaxis?

      predation as a behavior?

            Fig. 28.24, (Campbell and Reece, 2005) Gymnamoeba

Also movement of protists by pseudopods is a repeatable behavior (Fukui and Inoué 1997).

Plant Behaviors

Plant stomatal behavior

      Fig. 10.3, leaf cross section and stomate

      stomatal swelling opens pore, how does a plant cell swell?

            recall system we have seen before...

      if high carbon dioxide in leaf?

      if want to prevent water loss?

      This is a repeatable behavior, at cellular level, that is very adaptive for plants that use it.

Plant "sleep" movements
      Fig. 39.20, plant sleep
      these are done at night, involve movements of leaves and stems over night
            (note this chapter in the text is titled:
                  plant responses to internal and external signals)
            a repeatable response seen over time, a behavior?
            Yet the chapter does not call it a behavior.
      Desmodium gyrans sleep movement.jpg (Darwin 1881; pg 358)
            so these behaviors have long been known.
            keeps leaves warmer over night,
                  by just a few degrees, but that matters over the long term
      note anticipation of the dawn by this *Malvastrum* sp.,
            something is learnt and remembered here? Not well studied at all...
            fig. 8.2, (Simons, 1992) plant_sleep2.jpg

Tendril movements
      Tendrils are modified leaves, typically involved in wrapping around items for vine support
      In some cases this is growth, but in others see more than just movement...
            Changes in osmotic pressure in various cells leads to motion
      maple leaf passion flower tendril movement.jpg (Gray 1873; pg 12)
      In this plant see a sense of self versus non-self
            its tendrils will wrap about non-self items, but not about self,
                  even if it is another branch of the same plant
            mechanism for this sensing of self is currently unknown
            a useful behavior since support is better from non-self items than from self...

Rapid plant responses
      Mimosa pudica the sensitive plant
            Fig. 39.26, mimosa
            responds to light, touch, temperature
                  an action potential is sent from leaflet down petiole and stem
                      action potential moves through phloem
                habituates to each separately
                habituation in an animal is a type of learnt behavior
                so this plant displays a type of learning...

So these are reproducible, non-growth responses to stimuli. Have underlying mechanisms.
      I call them behaviors...

      Any objections?
      Implications: Animal biologists' focus on nerves and muscles as being needed for
            behavior, while important, may be obscuring a larger view of the study of
            behavior? Do animals also have such simple behaviors? I would say yes...

Why are Non-animal Behaviors ignored?

Why the sterotype that plants lack behaviors?

      Not due to lack of knowledge of them. Have been long known about these traits...

      Title Page Original.jpg (Darwin 1881)

      How Plants Behave title page.jpg (Gray 1873)

      but there has been an avoidance of studies in this area, why?

If you five years ago on this campus you took a plant physiology class, or a class on behavior, you

            would not not learn about plant behaviors

      (I know this because I talked to students who took such courses...)

      Focus was be on hormones, growth, and other non-behavioral aspects of plants.

Why did plant biologists steer away from this interesting area?

The 60's, and "Flower Power"

      Popular images of plants created a steriotype to which even scientists reacted

          the result has been a pulling back by scientists from an interesting field of study...

Work of Backster: Video: "The Green Machine" (start at 40 min, 50 sec)

      Cleve Backster

      Review what he did, and what claims he made...

      Backster1.jpg, title (Backster, 1968)

      Backster2.jpg, expt. setup (Backster, 1968)

      Backster3.jpg, results (Backster, 1968)

      Supposed evidence of plant's ability to sense emotions, and other items

          this became very popular

Popular reaction vs. scientific reaction

      plant_secretlife1.jpg, book cover, (Tompkins etal, 1973)

      plant_secretlife2.jpg, TOC, (Tompkins etal, 1973)

      Note the popular claims became extreme and fixed into the culture

          "flower power"   "flower children" ?

      In the 1970s if you went to a psychologist it would not be unusual for a plant with

          a set of electrodes to have been used as part of your session!

Result was that many academics feared to touch the topic of plant behaviors and plant senses.

      Considered an area for fringe nutcases, so funding hard to get.

      Instead focused on quantifiable hormones, that gave measureable growth responses

          this focus dominates plant physiology to this day...

Test of "ESP" in plants
       Cornell Undergraduates wanted to test Backster's work, senior thesis work...
       No plant physiologist on campus would help them.
          Roger Spanswick was a new plant electrophysiologist who they approached
              he told them to go work for the animal electrophysiologist,
              who already had tenure!
     Horowitz1.jpg, title (Horowitz etal, 1975)
     Horowitz2.jpg, data (Horowitz etal, 1975)
     Horowitz3.jpg, data (below) Horowitz etal, 1975)

"Table 2. Summary of +, -, and 0 scores used in binomial analysis. Data were pooled on the basis of no interaction between simultaneous recordings within groups of four plants.

Ejection	Number of scores		
	+	**-**	**0**
Brine shrimp (N= 60)	10	14	36
Water (N = 40)	6	8	26"

(Horowitz et al., 1975)

"We believe that we matched, and in several instances improved on, Backster's experimental techniques, such as controls, shielding, number of observations, methods of analysis, and number of shrimp killed per injection. We obtained no evidence of primary perception in plants. While the hypothesis will remain as an intriguing speculation one should note that only the limited published data of Backster support it."
    - Horowitz, Lewis, and Gasteiger, pg. 480, Science, 1975, vol. 189 -

According to Dr. Gasteiger, they had a difficult time getting this work published.
     Many plant physiologists thanked him for this work, which they dared not touch...

Result:
     By this view behavior is an emergent property of life, not just of animals.
        (note that some biologists may disagree with this view...)
     Non-animal behaviors are poorly studied.
     So the field of study of plant behaviors is fairly wide open field.
     Are there aspects of behavior that are being missed which are non-neuronal based?

And this field of study, especially in the plant area, is wide open...

Objectives:

Be able to contrast the text's definition of behavior with the definition presented in this lecture. How do the systems described for *Myxococcus xanthus*, protists, and for plants fit the definition of behavior given in this lecture?

Be able to contrast a behavioral response with a growth response. Which type of response might each of the following be: The formation of an anthrax endospore? The swimming of a euglena towards the light? The sleep movement of a bean plant? The courtship of stickleback fish? In each case consider what specific information you would need to have to confirm your answer.

Consider what is needed in order for a behavior to be displayed by an organism. What molecular and cellular mechanisms must be present? Consider if these mechanisms are only found in animals, and only in the nervous and muscle tissues of animals.

Needed overheads and items:

Fig. 11.3, *Myxococcus xanthus*
Fig. 27.9, Anthrax spores
Fig. 27.5, bacterial pili
Fig. 28.7, *Euglena*
Fig. 28.24, (Campbell and Reece, 2005) Gymnamoeba
Fig. 10.3, leaf cross section and stomate
Fig. 39.20, plant sleep
Desmodium gyrans sleep movement.jpg (Darwin 1881; pg 358)
Fig. 8.2, (Simons, 1992) plant_sleep2.jpg
maple leaf passion flower tendril movement.jpg (Gray 1873; pg 12)
Fig. 39.26, mimosa
Title Page Original.jpg (Darwin 1881)
How Plants Behave title page.jpg (Gray 1873)

Video: The Green Machine (start at 40 min, 50 sec into it)

Backster1.jpg, title (Backster, 1968)
Backster2.jpg, expt. setup (Backster, 1968)
Backster3.jpg, results (Backster, 1968)
plant_secretlife1.jpg, book cover, (Tompkins etal, 1973)
plant_secretlife2.jpg, TOC, (Tompkins etal, 1973)
Horowitz1.jpg, title (Horowitz etal, 1975)
Horowitz2.jpg, data (Horowitz etal, 1975)
Horowitz3.jpg, results from study by Horowitz etal (1975)

Bring in:
Tompkins and Bird (1973) text;
Copy of Backster (1968) paper;
Copy of Horowitz, Lewis, and Gasteiger (1975) paper.

References:

Adler J- 1969-Chemoreceptors in bacteria: Studies of chemotaxis reveal systems that detect attractants independently of their metabolism- Science 166: 1588-1597

Backster C- 1968-Evidence of a primary perception in plant life- International Journal of Parapsychology 10: 329-348

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Biology. Eighth edition. Pgs. 777-778, 821-824, 838, 842-843. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-Biology, 7th edition, Fig. 28.24. Benjamin Cummings Press. San Francisco, CA.

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Darwin C- 1881-The Power of Movement of Plants. 592 pgs. D. Appleton and Company. N.Y., N.Y.

Fukui Y., S. Inoué- 1997-Amoeboid movement anchored by eupodia, new actin-rich knobby feet in *Dictyostelium*- Cell Motility and the Cytoskeleton 36: 339-354

Gray A- 1873-Botany for Young People. Part II. How Plants Behave: How they move, climb, employ insects to work for them, &c. 46 pgs. Ivison, Blakeman, Taylor, and Company. N.Y., N.Y.

Hemmersbach R., E. Becker, W. Stockem- 1997-Influence of extremely low frequently electromagnetic fields on the swimming behavior of ciliates- Bioelectromagnetics 18: 491-498

Horowitz K.A., D.C. Lewis, E.L. Gasteiger- 1975-Plant "primary perception": Electrophysiological unresponsiveness to brine shrimp killing- Science 189: 478-480

Simons P- 1992-The Action Plant: Movement and nervous behaviour in plants. 323 pgs. Blackwell Press. Cambridge, MA.

Tompkins P., C. Bird- 1973-The Secret Life of Plants. 402 pgs. Harper & Row Publishers. N.Y., N.Y.

Velicer G.J., K.L. Stredwick- 2002-Experimental social evolution with *Myxococcus xanthus*- Antonie van Leeuwenhoek 81: 155-164

Related issues:

For a bit more on **plant movements** that I would call behaviors:

Blatt M.R., A. Grabov- 1997-Signal redundancy, gates and integration in the control of ion channels for stomatal movement- Journal of Experimental Botany 48: 529-537

Bynum M.R., W.K. Smith- 2001-Floral movements in response to thunderstorms improve reproductive effort in the alpine species *Gentiana algida* (Gentianaceae)- American Journal of Botany 88: (#6) 1088-1095

Holmes E., G. Gruenberg- 1965-Learning in plants?- The Worm Runner's Digest 7: 9-11

Kevan P.G- 1975-Sun-tracking solar furnaces in high arctic flowers: Significance for pollination and insects- Science 189: (#4204, 8/29) 723-726

Larson K.C- 2000-Circumnutation behavior of an exotic honeysuckle vine and its native congener: Influence on clonal mobility- American Journal of Botany 87: (#4) 533-538

Morillon R., D. Liénard, M.J. Chrispeels, J-P. Lassalles- 2001-Rapid movements of plants organs require solute-water cotransporters or contractile proteins- Plant Physiology 127: (#3) 720-723

Moshelion M., N. Moran- 2000-Potassium-efflux channels in extensor and flexor cells of the motor organ of *Samanea saman* are not identical. Effects of cytoplasmic calcium- Plant Physiology 124: 911-919

Roblin G- 1979-*Mimosa pudica*: A model for the study of the excitability in plants- Biological Reviews 54: 135-153

Bacteria have daily **circadian rhythms**, just as eukaryotes do, and this can alter their responses in repeatable ways.

Golden S.S., M. Ishiura, C.H. Johnson, T. Kondo- 1997-Cyanobacterial circadian rhythms- Annual Review of Plant Physiology and Plant Molecular Biology 48: 327-354

Poon A.C., J.E. Ferrell jr- 2007-A clock with a flip switch- Science 318: (#5851,11/2) 757-758

Rust M.J., J.S. Markson, W.S. Lane, D.S. Fisher, E.K. O'Shea- 2007-Ordered phosphorylation governs oscillation of a three-protein circadian clock- Science 318: (#5851, 11/2) 809-812

How **bacteria sense** environmental signals is also important for their behaviors.

Collier R.J- 2010-*Salmonella*'s safety catch- Science 328: (#5981, 5/21) 981-982

Kennis J.T.M., S. Crosson- 2007-A bacterial pathogen sees the light- Science 317: (#5841, 8/24) 1041-1042

Swartz T.E., T-S. Tseng, M.A. Frederickson, G. Paris, D.J. Comerci, G. Rajashekera, J-G. Kim, M.B. Mudgett, E.A. Splitter, R.A. Ugalde, F.A. Goldbaum, W.R. Briggs, R.A. Bogomolni- 2007-Blue-light-activity histidine kinases: Two-component sensors in bacteria- Science 317: (#5841, 8/24) 1090-1093

For more on **social bacteria** see:

Holloway M-2004-Talking bacteria- Scientific American 290: (#2) 34-35

Jelsbak L., L. Søgaard-Andersen- 2002-Pattern formation by a cell surface-associated morphogen in *Myxococcus xanthus*- Proceedings of the National Academy of Science (USA) 99: (#4) 2032-2037

Kearns D.B., A. Venot, P.J. Bonner, B. Stevens, G-J. Boons, L.J. Shimkets- 2001-Identification of a developmental chemoattractant in *Myxococcus xanthus* through metabolic engineering- Proceeding of the National Academy of Science (USA) 98: (#24) 13990-13994

Kolodkin-Gal I., R. Hazan, A. Gaathon, S. Carmeli, H. Engelberg-Kulka- 2007-A linear pentapeptide is a quorum-sensing factor required for mazEF-mediated cell death in *Escherichia coli*- Science 318: (#5850, 10/26) 652-655

Nudleman E., D. Wall, D. Kaiser- 2005-Cell-to-cell transfer of bacterial enter membrane lipoproteins- Science 309: (7/1) 125-127

Yu Y-T.N., X. Yuan, G.J. Velicer- 2010-Adaptive evolution of an sRNA that controls *Myxococcus* development- Science 328: (#5981, 5/21) 993

Here are reviews of social behavior in **amoeba**:

Gregor T., K. Fujimoto, N. Masaki, S. Sawai- 2010-The onset of collective behavior in social amoebae- Science 328: (#5981, 5/21) 1021-1025

Prindle A., J. Hasty- 2010-Stochastic emergence of groupthink- Science 328: (#5981, 5/21) 987-988

BIO 107   2010
Homework set #1   Name: _____
Due 9:00 am, Monday, June 21. (Text references are indicated for each question.)
       Points earned: _____ = _____ for course.
                                 5
_____
1.) (10 pts) (Lecture 1. BIO 1107 lab manual, chapter 1) Kettlewell did many other studies of selection on moths. In one study he noted that across the Shetland Islands for one moth species 97% of individuals in the northern islands were dark in color, while in the southern Shetlands Islands only 2% of individuals were observed to be dark in color. Kettlewell captured many of these moths, marked them and released them at a northern Shetland Island. After sufficient time he recaptured some of his marked moths. Table 1 has the release and recapturing numbers for his studies:

   Table 1. Released and recaptured moths of species *Amathes Glareosa* in a study done by Kettlewell in 1961.

Morph Type	Number Released	Number Recaptured	Percentage recaptured
Light	2089	95	
Dark	2260	132	
Total =			

a.) Do the calculations needed to complete table 1, and fill in the empty cells in it.
b.) Does the study outlined above represent an experimental or an observational study? Explain the reasoning behind your answer by noting how what Kettlewell did that is consistent with that type of study.

c.) Give appropriate statements of the null (Ho) and alternative (Ha) hypotheses for this study.

Ho:

Ha:

d.) Based on just these results what conclusion do you reach? Explain the reasoning behind your answer.

[Source: Kettlewell H.B.D- 1961-Selection experiments on melanism in *Amathes Glareosa* Esp. (Lepidoptera)- Heredity 16: 415-434]

2.) (6 pts) (Lecture 2. See pgs. 12-17.)
Some argue for the view that life is a "perfect machine" displaying the ultimate in order and stability, without errors or flaws. If this were true, would this have any consequences on the operation of natural selection-driven speciation as envisioned by Darwin? If so, state how, if not, describe why not. Describe the reasoning behind your answer.

3.) (11 pts) (Lecture 3. See pgs. 38-49, 60-66.)
In the following molecule circle an example of each of the following items and label it with the appropriate letter or number.

Chlorophyll *a*

a.) A carbonyl group.

b.) A methyl group.

c.) An ionic bond.

d.) A non-polar covalent bond.

e.) A polar covalent bond.

f.) This part of the molecule is hydrophobic.

g.) This part of the molecule is hydrophilic.

h.) An asymmetric carbon.

Also: Circle three carbons in this molecule that differ in oxidation states and label them from most reduced (1), middle reduced state (2), to relatively most oxidized (3) of the three carbons you select as examples.

3

4.) (10 pts) (Lecture 4. Chapter 5.)
Refer to the images below in answering the following questions.

a.) Which letters are items that are normally found in DNA?

_____

b.) To make a nucleotide, what other item would be needed that is not shown here?

_____

c.) Which letters are of items that would be a part of CTP?

_____

d.) Circle an example of just one amine group somewhere in the figure.

e.) Item G is what type of sugar?

_____

And is it an alpha- or a beta- form of that sugar?

_____

What specific feature of it supports your answer on whether it is the alpha- or beta- form?
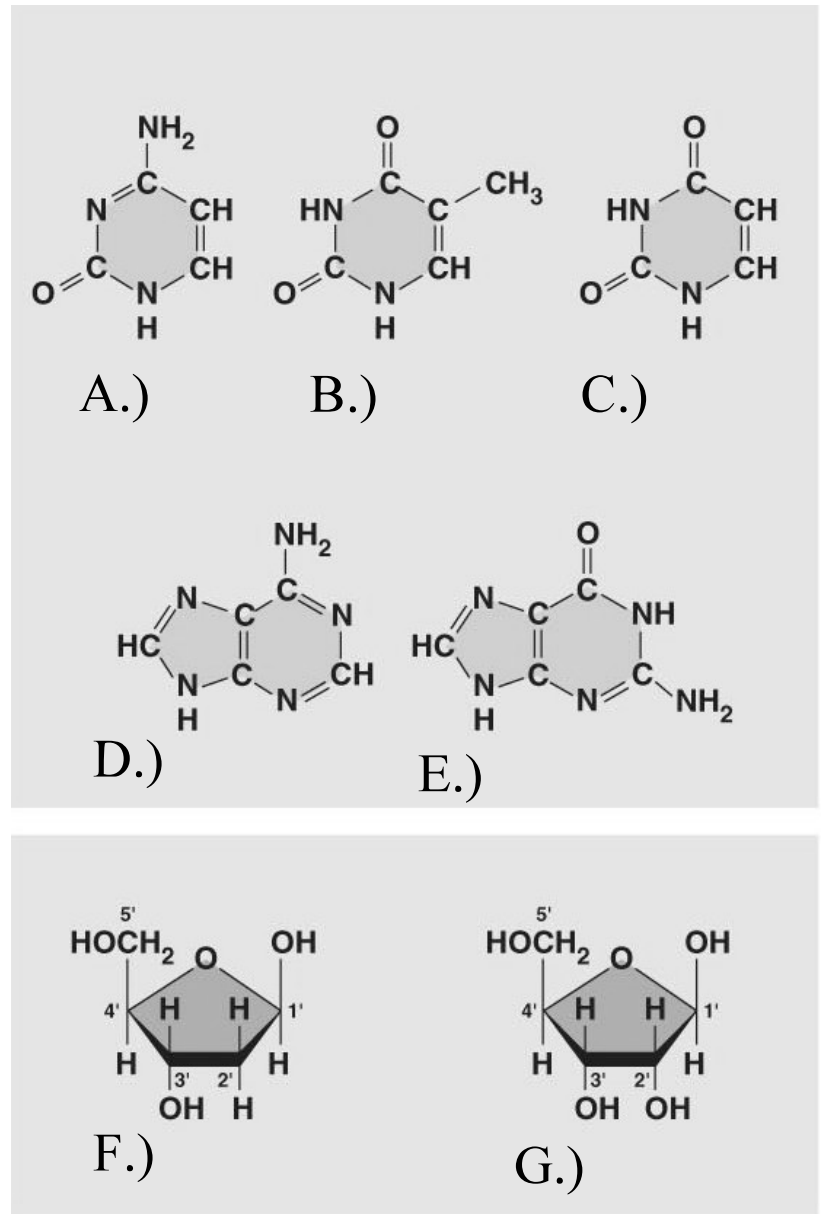
4

5.) (7 pts) (Lecture 5. Pgs. 146-153.)
The following figure shows the relative free energy state of reactants and products for a reversible reaction cycle. Two paths of the reaction (1 and 2) are indicated in the figure.
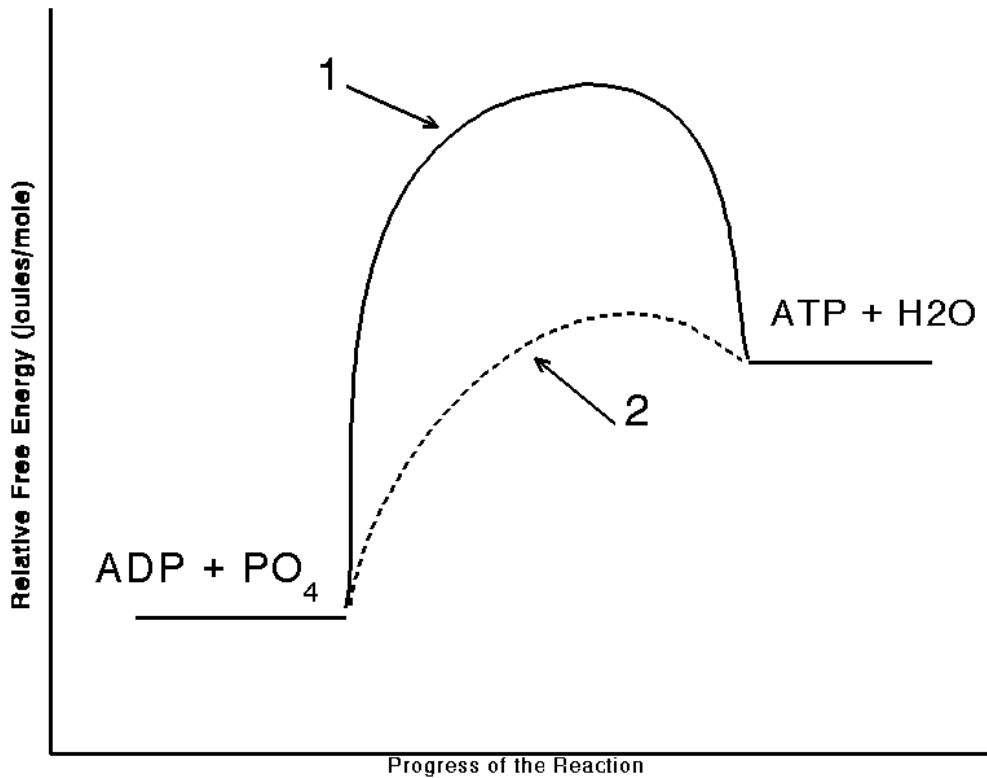
a.) Identify the direction in which this reaction would spontaneously occur by indicating the specific reactants and products (i.e. A + B → C + D) for this case..

b.) Which reaction pathway(s) has/have most likely not been catalyzed by an enzyme?

_____

c.) Of the two reaction paths shown:
   The one that occurs at the slowest rate is most likely _____ .
d.) On the figure draw a bracket to indicate the free energy ACTIVATION ENERGY barrier for an ENDERGONIC reaction, and label it AE.
e.) In the absence of an enzyme are ATP and water stable molecules or not? Support your answer with reference to relevant information from the figure.

6.) (8 pts) (Lecture 6. Pgs. 98-99, 112-118.)
Answer the following questions about the eukaryotic cytoskeleton.
a.) Name three types of proteins that make up the structure of the typical eukaryotic cytoskeleton, and for two of them name a specific motor protein that would work along the structure made up of that protein.

b.) List three important functions of the cytoskeleton and its associated proteins.

c.) The cytoskeleton, while present, is not as prominent in prokaryotes as it is in eukaryotes. What is a reason for how prokaryotes get by with much less cytoskeleton?

7.) (8 pts) (Lecture 7. See pgs. 768-770) Look at the
following figure showing a "U" tube with two solutions
(A and B) separated by a semi-permeable membrane that
allows water to pass, but not the sugar. Answer the
following questions about it.

Same concentration of sugar

a.) Initially solutions A and B are best described as:

    1.) Isoosmotic.
    2.) A is hyperosmotic to B.
    3.) A is hypoosmotic to B.

      Answer: _____

b.) Which solution (A or B) as shown in this figure has
water at an initially higher energy state? Identify the
specific form of the energy gradient that exists, and
which way will water flow as a result?

c.) Eventually the energies of the water on the opposite sides of this U tube will become equal.
Starting from the initial situation shown in the figure, describe what changes will happen to
solutions A and B in terms of their relative osmolarities and pressures as the energy gradient of
water between these two solutions moves to zero.

(Not for any points, but if you understand the above, you should now be able to look at figure 7.12 of the text and
understand how it is wrong.)

7

8.) (12 pts) (Lecture 11. Chapter 11.)
Examine fig. 11.16 (pg. 220 of our text). Identify from that figure the following items:

a.)	The signal.	
b.)	The type and location of the receptor of the signal.	
c.)	Three transduction elements present in this figure are:	
d.)	One transduction element that must be present for this system to work but which is NOT shown is:	
e.)	The final response indicated is:	
f.)	Two things that must be done to reset the transduction system shown here are:	

9.) (8 pts) (Lecture 8. See pgs. 167-172.)
For each of the following processes identify **all** of the net substrates that are used, and **all** of the net products that result, by one round of just that process.

Process	Net Substrates	Net Products
Glycolysis:		
Citric Acid cycle:		

10.) (11 pts) (Lecture 9. Pgs. 163-172.)

Rank the following reactions from that with the largest absolute value of net free energy change (1) to that with the lowest net free energy change (4). For each reaction, indicate the compartment(s) of a eukaryotic cell in which or across which each reaction normally occurs.

Reaction:	Rank of free energy change:	Compartment(s) in which it occurs:
Glyceraldehyde-3-phosphate + phosphate = 1,3-bisphosphoglycerate		
$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$		
$ADP + H_3PO_4 = ATP + H_2O$		
$NAD^+ + 2H = NADH + H^+$		

Are the reactants for the reaction you have ranked as having the largest free energy change highly stable or unstable? Describe the reasoning behind your answer.

11.) (9 pts) (Lecture 10. See chapters 9 and 10.)

Assume that the above represents part of a typical eukaryotic cell with just the membranes of the cell and of two organelles shown in this cross section. In the figure indicate using the appropriate letter(s) **all** the typical locations of each of the following as seen just in this part of this cell. Assume it is a warm sunny day and the cell is under normal aerobic conditions.

a.) The soluble compartment(s) that normally is(are) close to pH 7.
b.) The side of the appropriate membrane(s) where water is either destroyed or formed.
c.) The area(s) where starch would normally be made during the day.
d.) The membrane(s) in which chlorophyll is present.
e.) Location(s) where substrate-level phosphorylation normally occurs.
f.) The location(s) of any DNA in the structures shown.
g.) The membrane(s) across which the process of chemiosmosis occurs.
h.) Area(s) where carbon fixation occurs.

BIO 107   2010

Proposed Answers for Homework set #1.

Below are some possible answers to the questions in this homework set. Please note that other answers might receive full or partial credit.

Please look over the proposed answers both so that you can gain a sense of where I was headed with each question, and to give yourself some feedback on the issues raised by these questions.

Anyone who wishes more feedback may come see me about items in this homework set. Please see the course syllabus for information about how to request regrading of any lecture item.

BIO 107   2010
Homework set #1   Name: _____
Due 9:00 am, Monday, June 21. (Text references are indicated for each question.)
         Points earned: _____ = _____ for course.
                                5

1.) (10 pts) (Lecture 1. BIO 1107 lab manual, chapter 1) Kettlewell did many other studies of selection on moths. In one study he noted that across the Shetland Islands for one moth species 97% of individuals in the northern islands were dark in color, while in the southern Shetlands Islands only 2% of individuals were observed to be dark in color. Kettlewell captured many of these moths, marked them and released them at a northern Shetland Island. After sufficient time he recaptured some of his marked moths. Table 1 has the release and recapturing numbers for his studies:

Table 1. Released and recaptured moths of species *Amathes Glareosa* in a study done by Kettlewell in 1961.

Morph Type	Number Released	Number Recaptured	Percentage recaptured
Light	2089	95	*4.5%*
Dark	2260	132	*5.8%*
Total =	*4349*	*227*	

a.) Do the calculations needed to complete table 1, and fill in the empty cells in it.
b.) Does the study outlined above represent an experimental or an observational study? Explain the reasoning behind your answer by noting how what Kettlewell did that is consistent with that type of study.

This is an experimental study. By releasing marked moths Kettlewell was not merely observing things, but manipulating the situation. Such manipulation is what is done during an experimental study.

c.) Give appropriate statements of the null (Ho) and alternative (Ha) hypotheses for this study.

Ho: *There is no significant difference between the percentage of recaptured dark and light colored marked moths.*

Ha: *There is a significan difference between the percentage of recaptured dark and light colored marked moths.*

d.) Based on just these results what conclusion do you reach? Explain the reasoning behind your answer.

The results support the null hypothesis: There is no significant difference in the percentage of recaptured moths of these two colors. This implies that there was no difference in the selection that these two types of moths experienced over this time in this place.

[Source: Kettlewell H.B.D- 1961-Selection experiments on melanism in *Amathes Glareosa* Esp. (Lepidoptera)- Heredity 16: 415-434]

2.) (6 pts) (Lecture 2. See pgs. 12-17.)
Some argue for the view that life is a "perfect machine" displaying the ultimate in order and stability, without errors or flaws. If this were true, would this have any consequences on the operation of natural selection-driven speciation as envisioned by Darwin? If so, state how, if not, describe why not. Describe the reasoning behind your answer.

If individual members of a species were perfectly adapted to their environment and did not vary, then Darwinian speciation would likely not happen. There would be no variation, and no differences in fitness, upon which natural selection could operate.

3.) (11 pts) (Lecture 3. See pgs. 38-49, 60-66.)
In the following molecule circle an example of each of the following items and label it with the appropriate letter or number.

Chlorophyll a

a.) A carbonyl group.

b.) A methyl group.

c.) An ionic bond.

d.) A non-polar covalent bond.

e.) A polar covalent bond.

f.) This part of the molecule is hydrophobic.

g.) This part of the molecule is hydrophilic.

h.) An asymmetric carbon.

Also: Circle three carbons in this molecule that differ in oxidation states and label them from most reduced (1), middle reduced state (2), to relatively most oxidized (3) of the three carbons you select as examples.

4

4.) (10 pts) (Lecture 4. Chapter 5.)
Refer to the images below in answering the following questions.

a.) Which letters are items that are normally found in DNA?

    A, B, D, E, F

b.) To make a nucleotide, what other item would be needed that is not shown here?

    A phosphate group.

c.) Which letters are of items that would be a part of CTP?

    G, A

d.) Circle an example of just one amine group somewhere in the figure.

e.) Item G is what type of sugar?

    Ribose

And is it an alpha- or a beta- form of that sugar?

    Beta

What specific feature of it supports your answer on whether it is the alpha- or beta- form?

The hydrogen of carbon #1 is below the plane of the ring of the sugar, which is consistent with the beta form of this sugar.
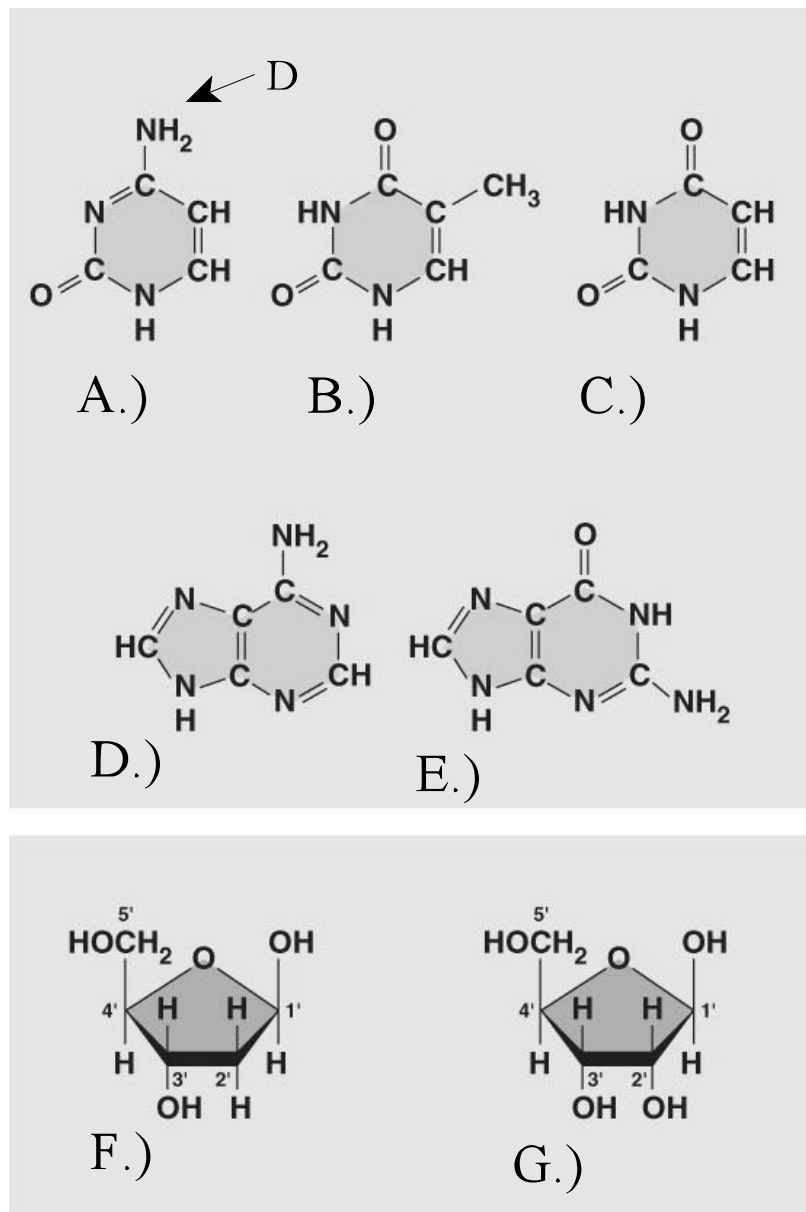
5.) (7 pts) (Lecture 5. Pgs. 146-153.)
The following figure shows the relative free energy state of reactants and products for a reversible reaction cycle. Two paths of the reaction (1 and 2) are indicated in the figure.

a.)   Identify the direction in which this reaction would spontaneously occur by indicating the specific reactants and products (i.e. A + B → C + D) for this case..

$$ATP + H_2O \ = \ ADP + PO_4$$
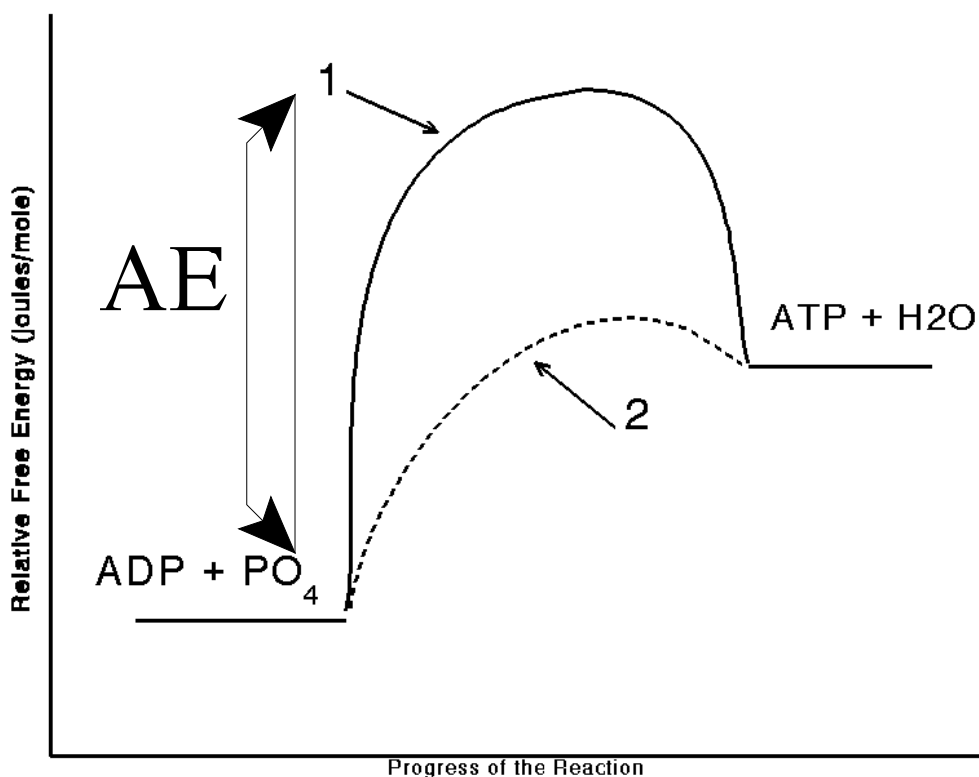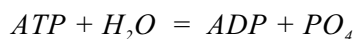
b.) Which reaction pathway(s) has/have most likely not been catalyzed by an enzyme?

1

c.) Of the two reaction paths shown:
    The one that occurs at the slowest rate is most likely   *1*   .
d.) On the figure draw a bracket to indicate the free energy ACTIVATION ENERGY barrier for an ENDERGONIC reaction, and label it AE.
e.) In the absence of an enzyme are ATP and water stable molecules or not? Support your answer with reference to relevant information from the figure.

They are stable. The activation energy barrier gives them stability as it is very rare for the energy needed to reach the peak of this barrier to be present in a molecule.

6.) (8 pts) (Lecture 6. Pgs. 98-99, 112-118.)
Answer the following questions about the eukaryotic cytoskeleton.
a.) Name three types of proteins that make up the structure of the typical eukaryotic cytoskeleton, and for two of them name a specific motor protein that would work along the structure made up of that protein.

keratin

actin - *myosin is a motor protein*

tubulin - *dynein is a motor protein*

b.) List three important functions of the cytoskeleton and its associated proteins.

Positioning organelles in the cell, intermediate filaments and microfilaments.

Generating and transmitting force through the cell, microfilaments and myosin.

Separation of chromosomes during mitosis, microtubules.

c.) The cytoskeleton, while present, is not as prominent in prokaryotes as it is in eukaryotes. What is a reason for how prokaryotes get by with much less cytoskeleton?

Being small, prokaryotes have enough internal mixing of their contents by diffusion. So they do not need to use their cytoskeleton to drive internal cytosolic streaming.

7.) (8 pts) (Lecture 7. See pgs. 768-770) Look at the following figure showing a "U" tube with two solutions (A and B) separated by a semi-permeable membrane that allows water to pass, but not the sugar. Answer the following questions about it.

a.) Initially solutions A and B are best described as:

    1.) Isoosmotic.
    2.) A is hyperosmotic to B.
    3.) A is hypoosmotic to B.

        Answer: *1*

Same concentration of sugar

b.) Which solution (A or B) as shown in this figure has water at an initially higher energy state? Identify the specific form of the energy gradient that exists, and which way will water flow as a result?

B. It has a higher pressure. So there is an energy gradient due to the pressure difference. The higher pressure gives solution B a higher energy state than solution A.

c.) Eventually the energies of the water on the opposite sides of this U tube will become equal. Starting from the initial situation shown in the figure, describe what changes will happen to solutions A and B in terms of their relative osmolarities and pressures as the energy gradient of water between these two solutions moves to zero.

Water will initially tend to move from solution B to A. A a result the solution in A will become a bit more dilute and the solution in B will become a bit more concentrated, producing a situation where B is hyperosmotic relative to A. Energy in this gradient will be balanced by the remaining pressure gradient with solution B having a higher pressure relative to solution A. Energy in these two gradients will ultimately balance, but note that the solute concentrations will not become equal.

(Not for any points, but if you understand the above, you should now be able to look at figure 7.12 of the text and understand how it is wrong.)
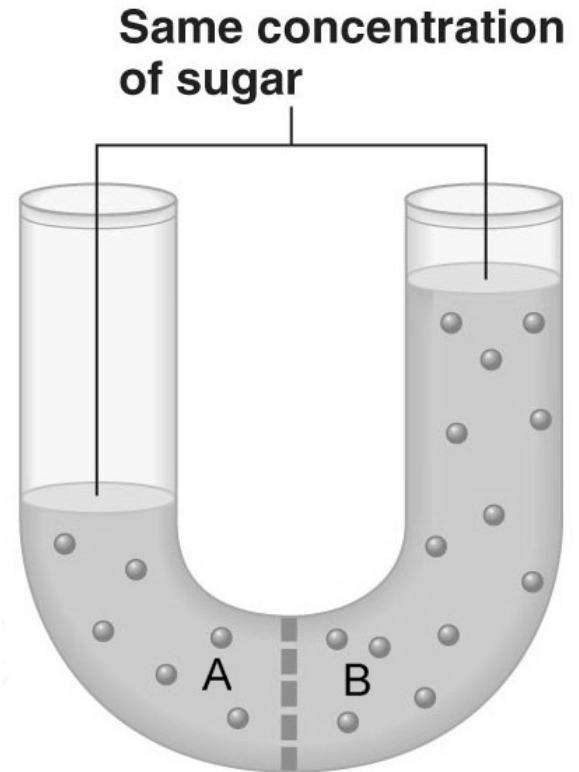
8.) (12 pts) (Lecture 11. Chapter 11.)
Examine fig. 11.16 (pg. 220 of our text). Identify from that figure the following items:

a.) The signal. *a mating factor*

b.) The type and location of the receptor of the signal. *G protein coupled receptor in the cell's plasma membrane.*

c.) Three transduction elements present in this figure are: *G protein, Fus3, Formin.*

d.) One transduction element that must be present for this system to work but which is NOT shown is: *Protein kinases are needed for the phosphorylation cascade.*

e.) The final response indicated is: *Change the cell's shape, producing a protrusion.*

f.) Two things that must be done to reset the transduction system shown here are: *Remove phosphate from P-Formin.*

The G protein must cleave GTP to GDP and phosphate.

9.) (8 pts) (Lecture 8. See pgs. 167-172.)
For each of the following processes identify **all** of the net substrates that are used, and **all** of the net products that result, by one round of just that process.

Process	Net Substrates	Net Products
Glycolysis:	*Glucose, ADP, phosphate, NAD^+*	*Pyruvate, water, ATP, NADH*
Citric Acid cycle:	*Acetyl-CoA, ADP, phosphate, NAD^+, FAD*	*CO_2, CoA, ATP, NADH, $FADH_2$*

10.) (11 pts) (Lecture 9. Pgs. 163-172.)

Rank the following reactions from that with the largest absolute value of net free energy change (1) to that with the lowest net free energy change (4). For each reaction, indicate the compartment(s) of a eukaryotic cell in which or across which each reaction normally occurs.

Reaction:	Rank of free energy change:	Compartment(s) in which it occurs:
Glyceraldehyde-3-phosphate + phosphate = 1,3-bisphosphoglycerate	2	*Cytosol*
$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$	1	*Cytosol, mitochondrial matrix*
$ADP + H_3PO_4 = ATP + H_2O$	4	*Cytosol, mitochondrial matrix*
$NAD^+ + 2H = NADH + H^+$	3	*Cytosol, mitochondrial matrix*

Are the reactants for the reaction you have ranked as having the largest free energy change highly stable or unstable? Describe the reasoning behind your answer.

These are stable molecules. There is a significant activation energy barrier between their bond states and the energy they have to have before they can undergo a reaction. This makes these molecules stable except under conditions that allow them to achieve that activation energy, like a fire.

11.) (9 pts) (Lecture 10. See chapters 9 and 10.)

Assume that the above represents part of a typical eukaryotic cell with just the membranes of the cell and of two organelles shown in this cross section. In the figure indicate using the appropriate letter(s) **all** the typical locations of each of the following as seen just in this part of this cell. Assume it is a warm sunny day and the cell is under normal aerobic conditions.

a.) The soluble compartment(s) that normally is(are) close to pH 7.
b.) The side of the appropriate membrane(s) where water is either destroyed or formed.
c.) The area(s) where starch would normally be made during the day.
d.) The membrane(s) in which chlorophyll is present.
e.) Location(s) where substrate-level phosphorylation normally occurs.
f.) The location(s) of any DNA in the structures shown.
g.) The membrane(s) across which the process of chemiosmosis occurs.
h.) Area(s) where carbon fixation occurs.

BIO 107   2010
Homework set #2   Name: _____
Due 9:00 am, Tuesday, June 29. (Text references are indicated for each question.)
       Points earned: _____ = _____ for course.
                                              5   (Please limit your answers to the spaces provided.)

1.) (6 pts) (Lecture 12. See chapter 28.) Based on the hypothetical phylogenic tree at the right of the protists Euglena, diatoms, Chlamydomonas and kelp answer the following questions.

a.) Does the vertical or the horizontal axis of this tree represent time?

_____

b.) Indicate with the letter "A" the position in the phylogenic tree of the most recent common ancestor of the four genera shown.

<u>Euglena</u>

<u>diatoms</u>   <u>Chlamydomonas</u>   <u>kelp</u>

multicellularity

Silica Cell Walls

Cellulosic Cell Walls

Flagella

c.) Based on **just** the information given in the above phylogenetic tree, what can be said about the characteristic(s) that this common ancestor likely had?

d.) Given what you know that goes beyond what is given in the phylogenic tree what is one additional characteristic that the common ancestor most likely had?

1

2.) (5 pts) (Lecture 13. See pgs. 772-773, 852-856, 887-888, 909-910, 969-971.)
Each of the following situations describes something that is done to try to over come a limitation imposed by diffusion. For each situation describe how the action taken by the organism is influencing the rate of diffusion, noting how that action overcomes a limit of diffusion?
a.) The essential ions taken up by the cells of a plant's roots are moved out of the root through the xylem up to its leaves.

b.) Trypsin in the lumen of our small intestine cleaves polypeptides into monomers.

c.) Our liver puts glucose, a monsaccarhide, into the blood serum rather than glycogen, a polysaccharide.

d.) Each active cell in our body is typically within less than a millimeter of a capillary bed.

e.) To recover more water from the urine cells lining out collecting ducts in the nephrons of our kidneys often deploy many more aquaporins to their membranes, which are channels specific for water.

3.) (10 pts) (Lecture 14. See text pgs. 121, 135-138, 884-890.)
Consider the mammalian small intestine, and answer the following questions about it.
a.) Identify two digestive enzymes that are made and deployed by cells of the small intestine, and note the specific location where each functions.

b.) Describe the role of tight junctions in the small intestines and identify where they occur.

c.) What is one way that the small intestine avoids being digested by pepsin passed along to it from the stomach?

d.) Describe the molecular system that would typically be used to move glucose out of the lumen of the small intestines and concentrate it into its epithelial cells.

4.) (10 pts) (Lecture 15. See text pages 906-911.)
Our capillary beds tend to have several features. Answer the following questions about them.
a.) Given that rapid exchange is often desired between the blood in the capillaries and the surrounding tissue is a higher or a lower blood pressure best to have at the capillaries? Why is this? Support your answer further by noting a specific feature of the capillaries and describing how it is consistent with your answer.

b.) Describe the main advantage to an organism whose capillaries have a higher total cross sectional area compared to the arteries that fed blood into them.

c.) In a portal system two capillary beds are in series. What is the consequence of this on the rate of flow of blood through the second capillary bed relative to the first? And what must differ across these two capillary beds to account for the difference in the rate of blood flow you indicate?

5.) (8 pts) (Lecture 16. See text pgs. 923-927.)
Consider human blood just exiting the capillaries of our lungs relative to blood just exiting the capillaries in another metabolically active tissue. Compare blood in these two locations by stating the typical values of each of the following items for each location.

Item to be compared:	Blood in lungs:	Blood in other tissue:
Blood pH:		
Blood partial pressure of oxygen gas (mm Hg):		
Blood partial pressure of carbon dioxide (mm Hg):		
Percent saturation of hemoglobin for oxygen gas:		

6.) (6 pts) (Lecture 17. See pgs. 785-797.)
Consider a field of wheat and the farmer.
Why would this farmer want to know the pH of the soil in the wheat fields?

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What two mineral nutrients are most likely to be in the fertilizer that is applied to the fields?

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This farmer would welcome bacteria that can carry out which metabolic processes in the soil?

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What is a metabolic process that is carried out by bacteria that a farmer would NOT like to see happen in the fields?

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7.) (9 pts) (Lecture 18. See our text, pgs. 963-968, 969-971.)
a.) Typically our blood in which area of our kidney has the higher blood pressure: The glomerulus or the Vasa recta? Describe how this is adaptive in terms of an associated function you identify.

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b.) There are many transport systems located in the epithelial cells of the proximal and distal convoluted tubules of our nephrons. Other than in the nephron, cells in what other structure of the kidney would need to have a high number of similar transport systems? Identify where these cells are located, and to where they are ultimately moving the items after they take them up into themselves.

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c.) Assume that an error occurs in gene expression so that the gene coding for aquaporin was not adequately expressed in the epithelial cells of our collecting ducts. What would be the consequences of this on each of the following items?

Volume of urine produced:

Blood osmolarity:

8.) (9 pts) (Lecture 19. See text pgs. 1105-1108.)
Consider the items shown to be involved in the cycle given below.
Label each of the following items in the figure, using the appropriate letter and a line drawn to the specific item it is meant to indicate. Note that some items will require just one label, some more.
   a.) Actin.
   b.) Myosin
   c.) The time(s) when the myosin active site is empty.
   d.) The time(s) when the myosin active site is occupied with a nucleic acid.
   e.) Stage that will predominate when ATP is lacking.
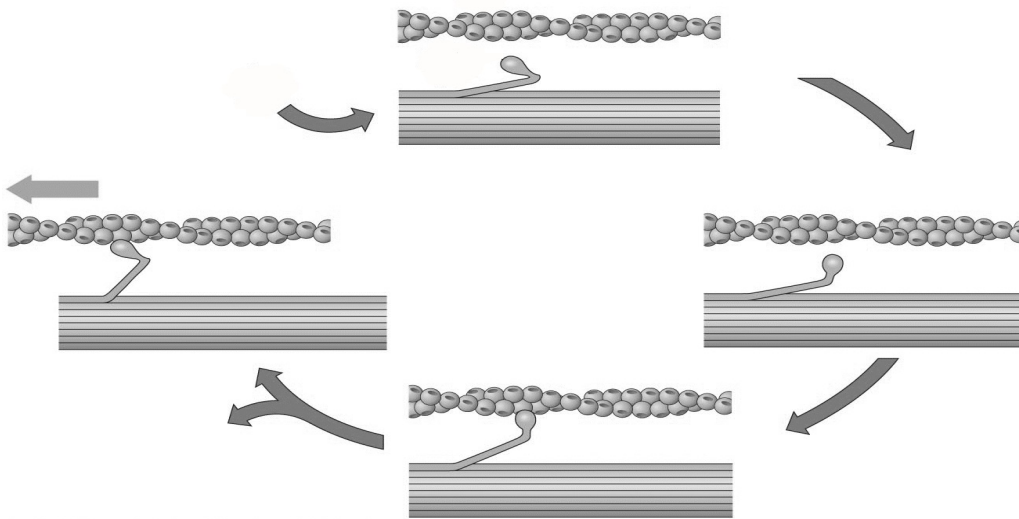
   f.) The above items are all parts of which specific structure?

   _____

   g.) The above cycle will typically occur when: (check all that apply)

      _____ Calcium ion channels of the sarcoplasmic reticulum have been activated.
      _____ The myosin ATPase activity is inhibited.
      _____ There is ATP available in the cell's cytosol.

6

9.) (5 pts) (Lecture 20. See pgs. 804-805, 809-811.)
Consider coconut plants.
Coconut fruits on their own will disperse by means of: _____ .
Describe a property of the coconut fruit and how it is consistent with this means of dispersal.

Coconuts, like typical flowering plants, produce pollen grains. Describe how the pollination of coconut flowers differs from dispersal of the coconut species.

10.) (8 pts) (Lecture 21. See pgs. 981-984.)
Consider the regulation of glucose concentration in the blood and its metabolism by cells of a mammal.
a.) If a mutation occurs in the gene that codes for a glucagon receptor in the cells of the liver such that the receptor does not function, what effect is that likely to have on what function of these cells?

b.) Describe a likely effect to this organism if the gene for the receptors of serum glucose in the beta cells of the pancreas mutates so that these receptors no longer function?

7

c.) Identify and describe the hormone whose action is antagonistic to glucagon. Note which endocrine gland secretes it, the conditions that stimulate the secretion of this other hormone, and note how the effects of this other hormone on what example organ results in its being antagonistic in effect to glucagon.

11.) (8 pts) (Lecture 22. See text pgs. 1007-1012.)
Contrast the affects on the anterior pituitary and hypothalamus of testosterone in mammalian males with that of estradiol (i.e estrogen) in mammalian females. Note similarities and differences, and the consequences these affects have in each sex.

12.) (8 pts) (Lecture 23. See pgs. 767-781.)

On a sunny day in a leaf the solution in the xylem tissue has the same water potential as the sap in the phloem tissue. This is typically the case since these two tissues are together in the vascular bundles of the leaf.

a.) Describe how the magnitude of the components of water potential differ between these two solutions even though they still have the same water potential.

b.) Obviously the plant is often playing an active role in altering the above water potential components. Of the components of water potential that you identified above which component in which tissue costs the plant the most to influence? Identify what that cost is by describing what the plant is paying to do that costs it so much (hint: think about actions at the subcellular level that the plant uses to manipulate this component of its water potential).

BIO 107   2010

Proposed Answers for Homework set #2.

Below are some possible answers to the questions in this homework set. Please note that other answers might receive full or partial credit.

Please look over the proposed answers both so that you can gain a sense of where I was headed with each question, and to give yourself some feedback on the issues raised by these questions.

Anyone who wishes more feedback may come see me about items in this homework set. Please see the course syllabus for information about how to request regrading of any lecture item.

Homework set #2   Name: _____

Due 9:00 am, Tuesday, June 29. (Text references are indicated for each question.)

      Points earned: _____ = _____ for course.
                                         5   (Please limit your answers to the spaces provided.)

1.) (6 pts) (Lecture 12. See chapter 28.)

Based on the hypothetical phylogenic tree at the right of the protists Euglena, diatoms, Chlamydomonas and kelp answer the following questions.

a.) Does the vertical or the horizontal axis of this tree represent time?

  vertical

b.) Indicate with the letter "A" the position in the phylogenic tree of the most recent common ancestor of the four genera shown.

c.) Based on **just** the information given in the above phylogenetic tree, what can be said about the characteristic(s) that this common ancestor likely had?

    It likely had flagella, lacked any cell wall, and was unicellular.

d.) Given what you know that goes beyond what is given in the phylogenic tree what is one additional characteristic that the common ancestor most likely had?

    It was eukaryotic.
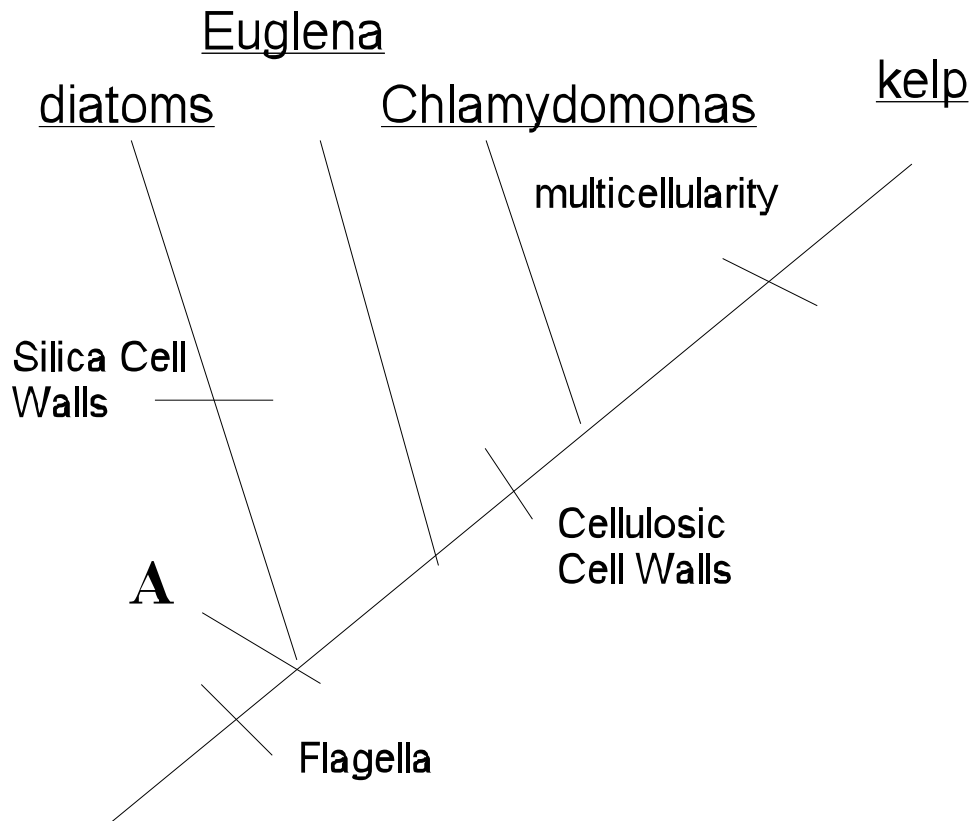
2.) (5 pts) (Lecture 13. See pgs. 772-773, 852-856, 887-888, 909-910, 969-971.)
Each of the following situations describes something that is done to try to over come a limitation imposed by diffusion. For each situation describe how the action taken by the organism is influencing the rate of diffusion, noting how that action overcomes a limit of diffusion?
a.) The essential ions taken up by the cells of a plant's roots are moved out of the root through the xylem up to its leaves.

This maintains the concentration gradient for this ion so that it can keep on diffusing up to the surface of the root cells. Otherwise it would accumulate in the roots.

b.) Trypsin in the lumen of our small intestine cleaves polypeptides into monomers.

This creates a large concentration of monomers so that their diffusion to the surface of cells is driven by it. The polypeptide concentration gradient would be much smaller.

c.) Our liver puts glucose, a monsaccarhide, into the blood serum rather than glycogen, a polysaccharide.

Glucose is smaller than glycogen, and smaller items diffuse faster than larger ones.

d.) Each active cell in our body is typically within less than a millimeter of a capillary bed.

Diffusion is faster over shorter distances than over longer ones. So keeping cells close to the capillary beds makes diffusion of items between them possible.

e.) To recover more water from the urine cells lining out collecting ducts in the nephrons of our kidneys often deploy many more aquaporins to their membranes, which are channels specific for water.

More water channels in the membrane lowers the resistance to the flow of water. This allows for a higher rate of osmosis out of the collecting duct.

3.) (10 pts) (Lecture 14. See text pgs. 121, 135-138, 884-890.)
Consider the mammalian small intestine, and answer the following questions about it.
a.) Identify two digestive enzymes that are made and deployed by cells of the small intestine, and note the specific location where each functions.

Disaccharidases: Located in the plasma membrane of epithelial cells.

Dipeptidases: Located inthe plasma membrane of epithelial cells.

b.) Describe the role of tight junctions in the small intestines and identify where they occur.

These are cell-cell connections between epithelial cells of the intestines. This binding blocks extracellular items from moving between the cells and so keeps the contents of the small intestine lumen and the body interstitial fluids distinct.

c.) What is one way that the small intestine avoids being digested by pepsin passed along to it from the stomach?

Pepsin needs an acidic pH to function, and the small intestine's lumen has a more neutral pH. This limits pepsin's digestive action when in the small intestine.

d.) Describe the molecular system that would typically be used to move glucose out of the lumen of the small intestines and concentrate it into its epithelial cells.

An ATPase pumps Na^+ out of the cell. This creates an electrochemical gradient for Na^+ across the membrane. This energy gradient is then coupled, via a Na^+/glucose symport, to the movement of glucose up its concentration gradient into the cell.

4.) (10 pts) (Lecture 15. See text pages 906-911.)
Our capillary beds tend to have several features. Answer the following questions about them.
a.) Given that rapid exchange is often desired between the blood in the capillaries and the surrounding tissue is a higher or a lower blood pressure best to have at the capillaries? Why is this? Support your answer further by noting a specific feature of the capillaries and describing how it is consistent with your answer.

A lower blood pressure in the capillary beds would tend to be better. The cells lining the capillaries are thin and structurally weak. If a high pressure gradient was applied across these cells it might rupture their connections and spill blood into the interstitial spaces. The thinness of the capillary cells is useful for exchange, and so the capillaries are limited to only being able to tolerate lower blood pressures.

b.) Describe the main advantage to an organism whose capillaries have a higher total cross sectional area compared to the arteries that fed blood into them.

Moving into this larger volume slows the forward flow of blood down. A slower flow rate of the blood allows more time for it to exchange items with the surrounding tissues.

c.) In a portal system two capillary beds are in series. What is the consequence of this on the rate of flow of blood through the second capillary bed relative to the first? And what must differ across these two capillary beds to account for the difference in the rate of blood flow you indicate?

The pressure gradient across the second capillary bed will be lower than that across the first capillary bed. This will mean that the flow of blood through the second capillary bed will tend to be slower than its rate through the first capillary bed.

5.) (8 pts) (Lecture 16. See text pgs. 923-927.)
Consider human blood just exiting the capillaries of our lungs relative to blood just exiting the capillaries in another metabolically active tissue. Compare blood in these two locations by stating the typical values of each of the following items for each location.

Item to be compared:	Blood in lungs:	Blood in other tissue:
Blood pH:	7.4	7.2
Blood partial pressure of oxygen gas (mm Hg):	100 mm Hg	40 mm Hg
Blood partial pressure of carbon dioxide (mm Hg):	40 mm Hg	46 mm Hg
Percent saturation of hemoglobin for oxygen gas:	100%	< 60%

6.) (6 pts) (Lecture 17. See pgs. 785-797.)
Consider a field of wheat and the farmer.
Why would this farmer want to know the pH of the soil in the wheat fields?

Many ions' solubility, and so their availability to the plants, is dependent on the pH of the soil solution.

What two mineral nutrients are most likely to be in the fertilizer that is applied to the fields?

Nitrogen and Phosphorus.

This farmer would welcome bacteria that can carry out which metabolic processes in the soil?

Nitrogen fixation.

What is a metabolic process that is carried out by bacteria that a farmer would NOT like to see happen in the fields?

Denitrification.

7.) (9 pts) (Lecture 18. See our text, pgs. 963-968, 969-971.)
a.) Typically our blood in which area of our kidney has the higher blood pressure: The glomerulus or the Vasa recta? Describe how this is adaptive in terms of an associated function you identify.

The higher blood pressure in the glomerulus helps to drive the filtration of the blood and so form the filtrate in the Bowman's capsule of the nephron. Without that high pressure not as much filtrate would be formed.

b.) There are many transport systems located in the epithelial cells of the proximal and distal convoluted tubules of our nephrons. Other than in the nephron, cells in what other structure of the kidney would need to have a high number of similar transport systems? Identify where these cells are located, and to where they are ultimately moving the items after they take them up into themselves.

The cells lining the capillaries around the nephrons would need to have these same transport systems so that they could take up these soluble items after the epithelial cells of the nephrons dump them into the intercellular solution. After taking up these items, these cells lining the capillaries would move these soluble items into the blood plasma.

5

c.) Assume that an error occurs in gene expression so that the gene coding for aquaporin was not adequately expressed in the epithelial cells of our collecting ducts. What would be the consequences of this on each of the following items?

Volume of urine produced: *Would be higher than normal.*

Blood osmolarity: *Would tend to stay higher than normal.*

8.) (9 pts) (Lecture 19. See text pgs. 1105-1108.)
Consider the items shown to be involved in the cycle given below.
Label each of the following items in the figure, using the appropriate letter and a line drawn to the specific item it is meant to indicate. Note that some items will require just one label, some more.

    a.) Actin.
    b.) Myosin
    c.) The time(s) when the myosin active site is empty.
    d.) The time(s) when the myosin active site is occupied with a nucleic acid.
    e.) Stage that will predominate when ATP is lacking.
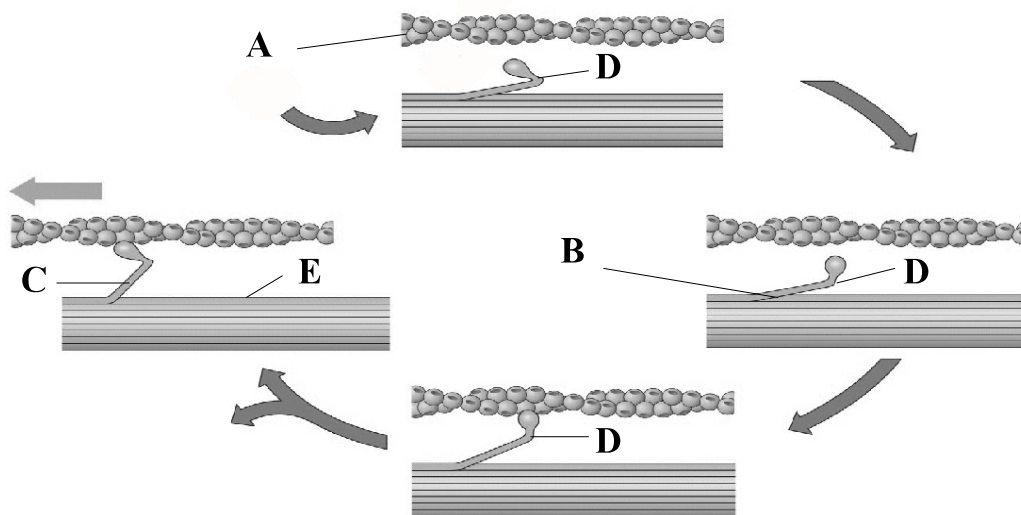
    f.) The above items are all parts of which specific structure?

        A sarcomere.

    g.) The above cycle will typically occur when: (check all that apply)

        X Calcium ion channels of the sarcoplasmic reticulum have been activated.
           The myosin ATPase activity is inhibited.
        X There is ATP available in the cell's cytosol.

9.) (5 pts) (Lecture 20. See pgs. 804-805, 809-811.)
Consider coconut plants.
Coconut fruits on their own will disperse by means of: *floating on the ocean*.
Describe a property of the coconut fruit and how it is consistent with this means of dispersal.

Their fibrous outer husk is buoyant. This helps these fruits to float well on the sea.

Coconuts, like typical flowering plants, produce pollen grains. Describe how the pollination of coconut flowers differs from dispersal of the coconut species.

Dispersal of fruits it attempting to find a new place for a new coconut plant to establish itself. It might disperse far from other members of its own species. In contrast, pollination involves moving a pollen grain from one coconut plant to another coconut plant. Thus pollination, if successful, must be limited to where coconuts are already located which makes it different from dispersal.

10.) (8 pts) (Lecture 21. See pgs. 981-984.)
Consider the regulation of glucose concentration in the blood and its metabolism by cells of a mammal.
a.) If a mutation occurs in the gene that codes for a glucagon receptor in the cells of the liver such that the receptor does not function, what effect is that likely to have on what function of these cells?

These cells would not detect glucagon, so they would not breakdown glycogen or release glucose out into the blood stream when they should.

b.) Describe a likely effect to this organism if the gene for the receptors of serum glucose in the beta cells of the pancreas mutates so that these receptors no longer function?

Then high blood sugar would not be detected, and so the beta cells would tend to not put out enough insulin when they should.

c.) Identify and describe the hormone whose action is antagonistic to glucagon. Note which endocrine gland secretes it, the conditions that stimulate the secretion of this other hormone, and note how the effects of this other hormone on what example organ results in its being antagonistic in effect to glucagon.

Glucagon tends to promote liver cells to breakdown glycogen and release glucose into the blood. In contrast, insulin stimulates liver cells to take up glucose from the blood and convert them to glycogen for storage. The secretion of insulin into the blood is stimulated by high blood sugar concentrations which are detected by receptors in the beta cells of the pancreas.

11.) (8 pts) (Lecture 22. See text pgs. 1007-1012.)

Contrast the affects on the anterior pituitary and hypothalamus of testosterone in mammalian males with that of estradiol (i.e estrogen) in mammalian females. Note similarities and differences, and the consequences these affects have in each sex.

In males, testosterone inhibits the release of GnRH from the hypothalamus, and the release of FSH and LH from the anterior pituitary. This causes the concentrations of FSH and LH that the testes encounters to be fairly stable, and so the production of sperm and testosterone by the testes tends to be steady over time.

In females, estrogen has a similar negative feedback affect as the above male situation during the mentral flow and early in the proliferative phase of the menstrual cycle. But later in the proliferative phase the higher concentrations of estrogen cause a positive feedback affect. This causes a rapid rise in the release of FSH and LH from the anterior pituitary, which in turn stimulates ovulation. Thus the situation in the female is not stable over time.

12.) (8 pts) (Lecture 23. See pgs. 767-781.)
On a sunny day in a leaf the solution in the xylem tissue has the same water potential as the sap in the phloem tissue. This is typically the case since these two tissues are together in the vascular bundles of the leaf.
a.) Describe how the magnitude of the components of water potential differ between these two solutions even though they still have the same water potential.

The pressure potential is negative (i.e. a tension) in the xylem solution, while in the phloem the pressure potential is typically very positive.
The osmotic potential in the xylem sap is slightly negative, while in the phloem the osmotic potential is very negative in value.
So even though the water potentials are the same in these two solutions, their components differ greatly.

b.) Obviously the plant is often playing an active role in altering the above water potential components. Of the components of water potential that you identified above which component in which tissue costs the plant the most to influence? Identify what that cost is by describing what the plant is paying to do that costs it so much (hint: think about actions at the subcellular level that the plant uses to manipulate this component of its water potential).

The osmotic potential of the phloem sap is very negative mainly due to the high osmolarity of this sap. Creating this high osmolarity involves mainly moving sucrose into the phloem sap, and this is very expensive. To do this sucrose movement is coupled to H^+ reentry into the phloem cells, via a H^+/sucrose symport system. The movement of sucrose up its concentration gradient is powered by the movement of H^+ down a greater electrochemical gradient. This H^+ electrochemical gradient is produced by the action of a H^+ pumping ATPase in the plasma membrane of the phloem cells. Thus the cost of loading the phloem is paid for by the hydrolysis of ATP.

BIO 107   2010
Homework set #3   Name: _____
Due by 9:00 am, Wednesday, July 7 (Text references are indicated for each question.)
        Points earned: _____ = _____ for course.
                                        5
_____
1.) (6 pts) (Lecture 24. See text pages 698-733.)
Answer the following questions.
a.) Of the groups given below, which one are humans NOT a member of? _____

        Amniotes   Mammals   Chordates   Deuterostomes
        Tetrapods   Lobe-finned fish   Monotremes   Craniates
        Gnathostomes

What is one trait that distinguishes humans from the group you identified?

| |
| |

b.) What is one of the above groups that humans are a member of? _____
Identify one trait that distinguishes this group from its closest relatives?

| |
| |

2.) (10 pts) (Lecture 27: See pgs. 1047-1057.)
Match the letter(s) for the part(s) of a typical mammalian neuron that would be expected to have
each of the following items. (Some items may have more than one part in which it is located, in
such cases indicate all the correct areas that apply.)

        D: dendrites   C: cell body   AH: axon hillock
        A: axon   AT: axon terminal

_____ Voltage-sensitive Ca^{+2} channels.

_____ Ligand-gated Na^+ channel.

_____ This region is often tightly covered by the membranes of another cell.

_____ Many synaptic vesicles are accumulated in this region.

_____ Voltage-sensitive K^+ channels.

3.) (8 pts) (Lecture 25. See pgs. 1112-1117.)
The figure shows the forelimb of a fox. For the ankle joint, indicate the following items in the figure by drawing a line to a specific point and labeling it with the appropriate letter. (7 pts)

a.) The insertion point of the extensor
        muscle.
b.) The pivot point of the joint.
c.) The output point of force applied
        through this joint.
d.) A tendon of the extensor muscle.

What are the input (S_i) and output (S_o) distances relative to this ankle joint, as shown in this figure for this extensor muscle?

Input distance: _____
Output distance: _____

What is the speed mechanical advantage (SA) of this ankle joint for this extensor muscle?

        SA: _____

4.) (10 pts) (Lecture 26: See pgs. 97, 405.)
For each of the molecules listed below note two properties it has. Also, based on one of the properties you identify, note one technique that could be used to begin to separate this molecule out of a sample of many molecular types.

Molecule:	Properties and Technique:
ribosomal RNA	
Na^+/K^+-ATPase	
chlorophyll A	

Select one of the molecules from the previous page , (give its name _____), and for it identify:

A likely organism from which it could be extracted, and where in that organism it would likely be found:

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Another specific molecule in the same molecular class that shares the properties you noted for this molecule.

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Identify a technique that might be used to separate these two molecules of this same class. Describe how this technique would achieve this separation.

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5.) (10 pts) (Lecture 28. See pages 1056-1061 and 1078-1080 of the text.)
Consider a chemical synapse.
a.) Identify two membrane spanning proteins that are likely to be present in the pre-synaptic neuron but unlikely to be present in the nearby membrane of the post-synaptic neuron. Describe the function of each.

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b.) Identify one membrane spanning protein that is likely to be in the post-synaptic neuron near to this synapse, but unlikely to be in the nearby membrane of the pre-synaptic neuron. Describe its function.

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c.) Describe one change that could make this synapse's influence stronger.

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d.) After this synapse is used, it must recover so that it can be used again. What are three things that would need to be done to return it a ready-to-use state? Note where in the synapse each item is that you identify.

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6.) (9 pts) (Lecture 29. See pgs. 1064-1069.)
Refer to the following figure showing a human reflex response.
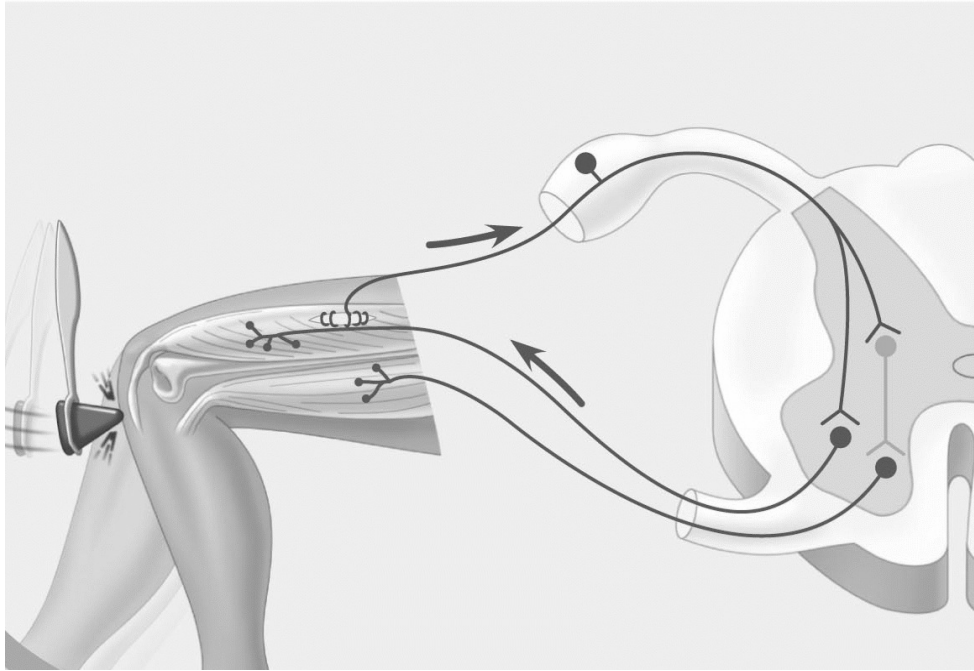
a.) There are four neurons indicated in the figure. Label each with a line and the appropriate letter to indicate its type.

   S: Sensory neuron.   M: Motor neuron.   I: Interneuron.

b.) Which type of neuron shown in the figure is most likely to also have a synapse onto an interneuron that extends to the brain?

_____
c.) What part of the central nervous system is shown in this figure?

_____
d.) Circle the location(s) of all the synapses onto a motor neuron shown in this figure.

e.) If the segment of the spine that is shown here is in the thoracic area, then in addition to the neurons shown in this figure, there would also be neurons that are part of the
      Sympathetic   **Parasympathetic** division (circle your choice). If those neurons extended to the heart, what likely affect would they have when activated?

7.) (9 pts) (Lecture 30. See pgs. 1087-1105.)
The image shows some of the events in a sensory cell involved in a sense in mammals. Answer the following questions concerning it.

a.) The transduction events shown here involves the closing of a K^+ ion channel (#3). This would most likely result in a

_____ of the plasma membrane's potential, and for this to influence the activity of the Na^+ channel (#4) this Na^+ channel must be:

     a.) mechanosensitive.
     b.) a ligand-gated channel.
     c.) a voltage-sensitive channel.

       Answer: _____

b.) Identify three aspects of events shown in this cell that are similar to events in the axon terminal of a typical mammalian neuron.

c.) What is the final response of **this** sensory cell that is induced by detection of a molecule by its receptor?

d.) Based on what is shown in this figure, what are three things that would have to be reset in order for this cell to be used again to respond to another chemical signal it detects?
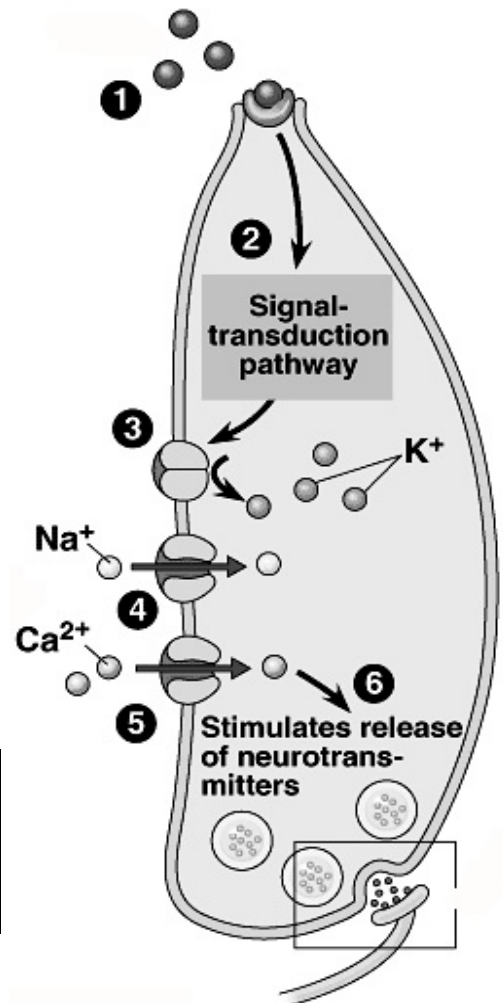
8.) (8 pts) (Lecture 31. See pgs. 1087-1096.)

Figure 50.9 (on pg. 1094 of our text) describes the functions of a sensory hair cell in a vertebrate. Consider the following questions concerning the situation shown in this figure. (8 pts)

a.) What specific feature is being detected and what class of sensory receptor is most likely to be involved in this case?

b.) The brain perceives the feature you identified above, but the sensory neuron shown in this figure encodes this information in what form to get it from the end of its cell next to the hair cell to the end of the sensory neuron that extends into the brain?

c.) Is this system a phasic or a tonic system? Explain the reasoning behind your answer by noting distinctive features shown in this figure.

d.) Another feature that this organism might want to monitor is whether the temperature of this sensory cell changes rapidly. If such a sensory system was set up would it be a phasic or a tonic system? Also, would the sensory neuron shown in this figure be able to also carry this information as well as what it is currently carrying? If so, suggest how it could do this. If not, then what would be needed to carry this information to the brain.

BIO 107   2010

Proposed Answers for Homework set #3.

Below are some possible answers to the questions in this homework set. Please note that other answers might receive full or partial credit.

Please look over the proposed answers both so that you can gain a sense of where I was headed with each question, and to give yourself some feedback on the issues raised by these questions.

Anyone who wishes more feedback may come see me about items in this homework set. Please see the course syllabus for information about how to request regrading of any lecture item.

BIO 107   2010
Homework set #3   Name: _____
Due by 1:00 pm, Wednesday, July 7 (Text references are indicated for each question.)
      Points earned: _____ = _____ for course.
                                 5

1.) (6 pts) (Lecture 24. See text pages 698-733.)
Answer the following questions.
a.) Of the groups given below, which one are humans NOT a member of? *Monotremes*

Amniotes	Mammals	Chordates	Deuterostomes
Tetrapods	Lobe-finned fish	Monotremes	Craniates
Gnathostomes			

What is one trait that distinguishes humans from the group you identified?

Humans are placental mammals, monotremes do not have a placenta.

b.) What is one of the above groups that humans are a member of? *Mammals*
Identify one trait that distinguishes this group from its closest relatives?

Mammals have hair.

2.) (10 pts) (Lecture 27: See pgs. 1047-1057.)
Match the letter(s) for the part(s) of a typical mammalian neuron that would be expected to have each of the following items. (Some items may have more than one part in which it is located, in such cases indicate all the correct areas that apply.)

D: dendrites   C: cell body   AH: axon hillock
A: axon   AT: axon terminal

AT   Voltage-sensitive Ca^{+2} channels.

D   Ligand-gated Na^+ channel.

A   This region is often tightly covered by the membranes of another cell.

AT   Many synaptic vesicles are accumulated in this region.

A, AT, AH   Voltage-sensitive K^+ channels.

3.) (8 pts) (Lecture 25. See pgs. 1112-1117.)
The figure shows the forelimb of a fox. For the ankle joint, indicate the following items in the figure by drawing a line to a specific point and labeling it with the appropriate letter. (7 pts)

a.) The insertion point of the extensor
       muscle.
b.) The pivot point of the joint.
c.) The output point of force applied
       through this joint.
d.) A tendon of the extensor muscle.

What are the input (S_i) and output (S_o) distances
relative to this ankle joint, as shown in this
figure for this extensor muscle?

Input distance: _1.0 cm_
Output distance: _5.5 cm_

What is the speed mechanical advantage (SA) of this ankle joint for this extensor muscle?

        SA: _5.5_

4.) (10 pts) (Lecture 26: See pgs. 97, 405.)
For each of the molecules listed below note two properties it has. Also, based on one of the properties you identify, note one technique that could be used to begin to separate this molecule out of a sample of many molecular types.

        Molecule: Properties and Technique:

  ribosomal RNA   *A negative charge - Can be used by electrophoresis to separate it.*
                         A large size for a molecule.

  Na^+/K^+-ATPase   *Hydrophobic, as it has a membrane spanning region.*
                         Density, proteins have different densities from other molecules. So
                         differential centrifugation would be one option to separate it.

  chlorophyll A   *Are hydrophobic, due to the hydrocarbon tail, and this could allow the*
                         use of thin layer chromatography to separate it.
                         It has a small size compared to some molecules.

Select one of the molecules from the previous page , (give its name *ribosomal RNA*), and for it identify:

A likely organism from which it could be extracted, and where in that organism it would likely be found:

This would occur in the cytosol of a bacterium such as E. coli.

Another specific molecule in the same molecular class that shares the properties you noted for this molecule.

DNA

Identify a technique that might be used to separate these two molecules of this same class. Describe how this technique would achieve this separation.

DNA tends to be much larger than ribosomal RNA. Thus we could use size-exclusion chromatography to separate them. If the pores are large enough the ribosomal RNA will enter them and travel slowly through the column, while the much larger DNA would be excluded and pass quickly through the column.

5.) (10 pts) (Lecture 28. See pages 1056-1061 and 1078-1080 of the text.)
Consider a chemical synapse.
a.) Identify two membrane spanning proteins that are likely to be present in the pre-synaptic neuron but unlikely to be present in the nearby membrane of the post-synaptic neuron. Describe the function of each.

Voltage-sensitive Ca^{+2} channels. It would detect the depolarization of the membrane potential and then open to allow Ca^{+2} to enter the cell to induce exocytosis.

Voltage-sensitive Na^+ channels. These are needed to generate the action potential in the axon terminus. Once past threshold these channels let Na^+ enter the cell and produce the initial part of the action potential.

b.) Identify one membrane spanning protein that is likely to be in the post-synaptic neuron near to this synapse, but unlikely to be in the nearby membrane of the pre-synaptic neuron. Describe its function.

A ligand-gated K^+ channel. It will detect a neurotransmitter in the synaptic cleft, and then allow K^+ to leave the cell, generating an inhibitory post-synaptic potential (IPSP).

c.) Describe one change that could make this synapse's influence stronger.

More receptors per area of the post-synaptic side of this synapse might be deployed. This would allow more ion movement to occur, making the amplitude of the IPSP stronger.

d.) After this synapse is used, it must recover so that it can be used again. What are three things that would need to be done to return it a ready-to-use state? Note where in the synapse each item is that you identify.

Synaptic vesicles were used, so more vesicles must be made and filled with neurotransmitter molecules.

The neurotransmitters in the synaptic cleft must be removed so that the receptors in the post-synaptic membrane do not encounter and bind them any more.

The calcium ions that entered the axon terminal cytosol must be removed. Most likely an active transport system will pump these ions back out of the cell.

6.) (9 pts) (Lecture 29. See pgs. 1064-1069.)
Refer to the following figure showing a human reflex response.
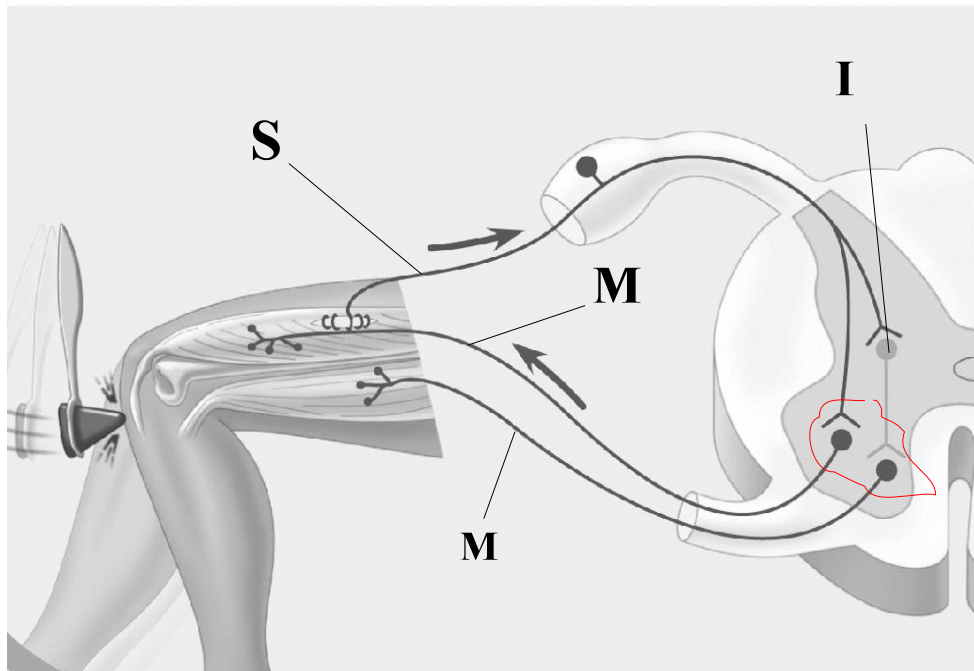
a.) There are   four
neurons indicated in the figure. Label each with a line and the appropriate letter to indicate its
type.

        S: Sensory neuron.   M: Motor neuron.   I: Interneuron.

b.) Which type of neuron shown in the figure is most likely to also have a synapse onto an
interneuron that extends to the brain?

        The sensor neuron.
c.) What part of the central nervous system is shown in this figure?

        The spinal cord.
d.) Circle the location(s) of all the synapses onto a motor neuron shown in this figure.
                        (Circled in red.)
e.) If the segment of the spine that is shown here is in the thoracic area, then in addition to the
neurons shown in this figure, there would also be neurons that are part of the
        Sympathetic   **Parasympathetic**   division (circle your choice). If those neurons
extended to the heart, what likely affect would they have when activated?

  It would accelerate the rate of the heart beat.

7.) (9 pts) (Lecture 30. See pgs. 1087-1105.)
The image shows some of the events in a sensory cell involved in a sense in mammals. Answer the following questions concerning it.

a.) The transduction events shown here involves the closing of a K^+ ion channel (#3). This would most likely result in a

___depolarization___ of the plasma membrane's potential, and for this to influence the activity of the Na^+ channel (#4) this Na^+ channel must be:

    a.) mechanosensitive.
    b.) a ligand-gated channel.
    c.) a voltage-sensitive channel.

       Answer: _C._

b.) Identify three aspects of events shown in this cell that are similar to events in the axon terminal of a typical mammalian neuron.

 A change in membrane potential.

 A rise in the cytosolic concentration of calcium ions.

 Induction of exocytosis of ligand-containing vesicles.

c.) What is the final response of **this** sensory cell that is induced by detection of a molecule by its receptor?

 The release of ligands into a synapse it has with a sensory neuron.

d.) Based on what is shown in this figure, what are three things that would have to be reset in order for this cell to be used again to respond to another chemical signal it detects?

 The K^+ channel (item #3) will have to be opened again.

 The Na^+ channel (item #4) will have to be closed.

 The Ca^{+2} channel (item #5) will have to be closed.
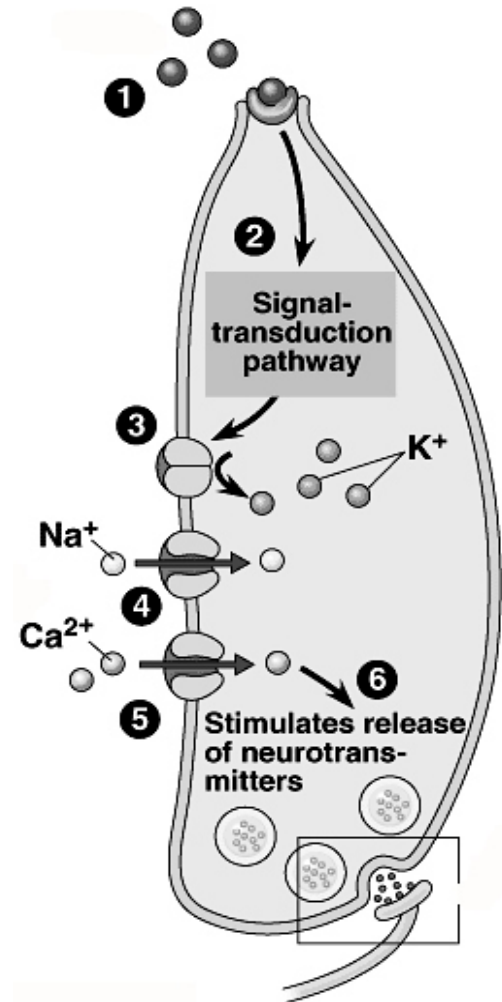
6

8.) (8 pts) (Lecture 31. See pgs. 1087-1096.)
Figure 50.9 (on pg. 1094 of our text) describes the functions of a sensory hair cell in a vertebrate. Consider the following questions concerning the situation shown in this figure. (8 pts)

a.) What specific feature is being detected and what class of sensory receptor is most likely to be involved in this case?

It detects the direction of the bending of the "hairs" of this cell. This most likely involves a type of mechano-sensitive receptor.

b.) The brain perceives the feature you identified above, but the sensory neuron shown in this figure encodes this information in what form to get it from the end of its cell next to the hair cell to the end of the sensory neuron that extends into the brain?

It is encoded in terms of the frequency of action potentials sent along the cell to the brain.

c.) Is this system a phasic or a tonic system? Explain the reasoning behind your answer by noting distinctive features shown in this figure.

It is a tonic system. Once bent the freqency of action potentials either drops, or rises, compared to the resting state. There is no drop off in the action potential frequency over time after the initial bending, as would be expected with a phasic system.

d.) Another feature that this organism might want to monitor is whether the temperature of this sensory cell changes rapidly. If such a sensory system was set up would it be a phasic or a tonic system? Also, would the sensory neuron shown in this figure be able to also carry this information as well as what it is currently carrying? If so, suggest how it could do this. If not, then what would be needed to carry this information to the brain.

This cell could use a phasic system to detect a change in temperature, and how long since a certain temperature was reached. To do this this cell would need a temperature sensor, an additional transduction system, that would induce vesicle exocytosis at a new synapse with another sensory neuron in response to temperature changes. The one sensory neuron shown in this figure can not carry two types of information down its length. A second sensory neuron would be needed to carry this different type of information, and it would likely go to a different part of the brain than does the sensory neuron shown in this figure.

BIO 107   Exam #1   Summer 2010

Tuesday, June 22   Name: _____

     Points earned: _____ = _____ for course.
                              70

Please keep your answers in the spaces provided, as answers outside of there will not be read!
Good luck!

1.) Describe the key differences between inductive and deductive reasoning. (2 pts)

2.) Which of the following would be expected to be a characteristic of a good scientific model?
(Check all that apply.)   (5 pts)

_____ It can explain all aspects relating to the topic(s) it covers.
_____ Derive from it testable and potentially refutable predictions.
_____ Be able to prove it based on collected sets of data.
_____ Have clearly stated sets of its assumptions and limits of its applicability.
_____ Be able to modify it if new data or phenomena demand it.

3.) A researcher is testing the effects of a new drug on blood pressure. He administers what he
thinks is an effective dose of the drug to fifty adult mice by injecting them with a solution of
artificial blood serum containing it, and he also gives an equal volume of just artificial blood
serum to fifty other adult mice. He then monitors blood pressure of each mouse over time. Give
an appropriate statement for the null and alternative hypotheses for the context of this experiment.
(4 pts)

Null hypothesis:

Alternative hypothesis:

4. Identify a structure found in life (as-we-know-it), and identify a major functional feature of life that directly depends on this structural element. (3 pts)

Structure:

Its Function:

5.) Which is LEAST supportive of the current model of biological evolution? (1 pt)

    a.) Individual members of a species have various traits.
    b.) Instead of being tens of thousands of years old the earth is actually billions of years old.
    c.) Genes are capable of being expressed over and over again.
    d.) The number of young produced by many species exceeds the number that a local area can support.
    e.) Individuals with some traits are more likely to survive to reproduce than individuals with different traits.

    Answer: _____

6.) From most biologists' point of view, which is the BEST reason for why most species are not perfectly adapted to their habitat? (2 pts)

    a.) They have not had enough time to evolve into a most fit state.
    b.) Species are fixed and are not able to change to achieve better characteristics.
    c.) There is rarely competition for resources between species, therefore each can have less than perfectly adaptive sets of characteristics.
    d.) Environmental shifts occur faster than natural selection can screen for the most fit combination of traits for survival in an area.
    e.) The traits that species acquire are often not inherited by the next generation.

    Answer: _____

7.) Rank the following types of interactions from strongest (1) to weakest (4). (4 pts)

_____ Between two carbon atoms in a molecule.
_____ Between a hydrogen atom in one molecule and an oxygen in another molecule.
_____ Between two hydrogen atoms found in two fat molecules.
_____ Between the hydrogen and an oxygen atom in a carboxyl group when placed in a
    solution at pH 2.

8.) When water vaporizes the bonds that are disrupted are mainly: (1 pt)

   a.) non-polar covalent bonds.
   b.) ionic bonds with other water molecules.
   c.) polar covalent bonds between oxygen and hydrogen atoms.
   d.) hydrogen bonds between different water molecules.
   e.) hydrophobic bonds.

       Answer: _____

9.) The figure at the right represents a monomer of chitin, a polymer common to insects and fungi. Answer the following concerning it: (7 pts)

a.) How many asymmetric carbons are present in this molecule?

    _____

b.) Identify the names (not the chemical symbols, but the actual names) of three types of functional groups found in this molecule.

    _____

    _____

    _____

c.) Use the appropriate chemical symbols to identify one example from chitin of each of the following, and **circle** both of your examples in the structure of the molecule.

A polar covalent bond occurs between: _____ .

A non-polar covalent bond occurs between: _____ .

3
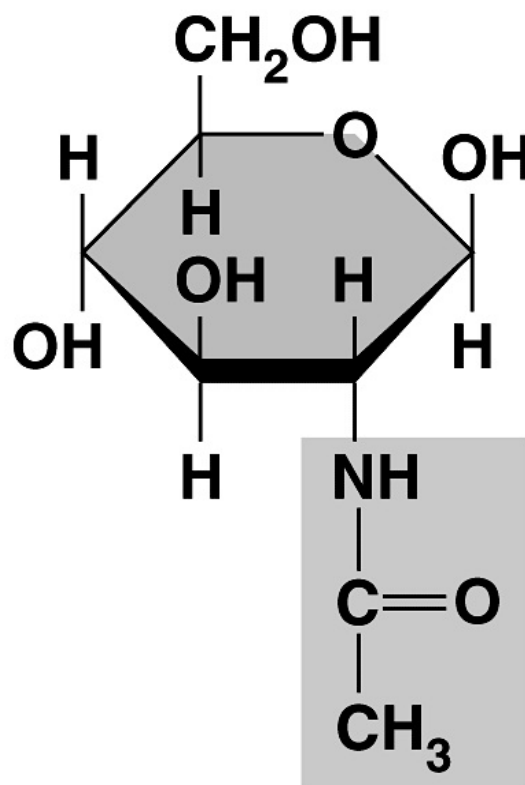
10.) The molecular formula for alanine (an amino acid) is $C_3H_7N_1O_2$. If five alanines were joined by peptide bonds to form a linear penta-peptide, then the molecular formula of that peptide would be: (1 pt)

a.) $C_{15}H_{35}N_5O_{10}$ b.) $C_3H_7N_1O_2$
c.) $C_{15}H_{33}N_5O_9$ d.) $C_{15}H_{27}N_5O_6$
e.) $C_{15}H_{47}N_5O_{14}$

        Answer: _____

11.) Complete the following with the best word or phrase. (2 pts)
Unlike carbohydrates, phospholipids are typically found to be a part of the

_____ of a cell. This is mainly due to the fact that these lipids are

typically _____ while most carbohydrates are not.

12.) Which BEST describes a unique aspect of quaternary structure compared to the other levels of protein structure? (1 pt)

a.) The sequence of amino acids within the polypeptide can have a large influence on the final configuration of the protein.
b.) Hydrogen bonds are very important for the folding of a polypeptide into alpha helices.
c.) The interaction of two or more polypeptides can influence the final shape and function of the resulting protein.
d.) Ionic bonds between various amino acid side groups are important only in quaternary structure.
e.) In quaternary structure a peptide bond is formed between the N-terminal amino acid of one polypeptide and the C-terminal amino acid of another polypeptide.

        Answer: _____
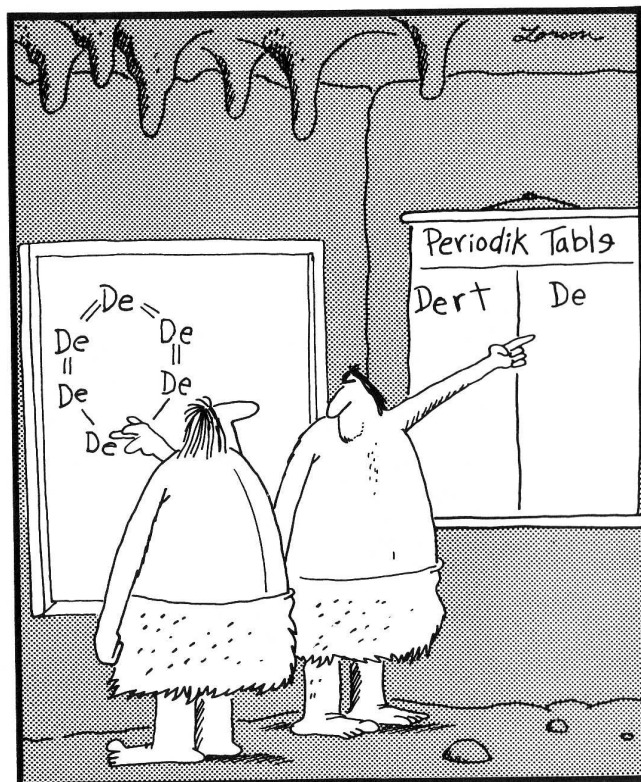
Early chemists describe the first dirt molecule.

13.) A sucrase enzyme adds water to sucrose to cleave it into fructose and glucose. This enzyme has a V_{max} of 3.5 μmoles of product per milligram of protein per minute, and a K_m for sucrose of 15 mM. A reaction system of 10 mL volume has one milligram of sucrase and 60 mM sucrose present. If a total reaction rate of 10.5 μmoles of product per minute is desired this can most easily be achieved by doing which of the following to this reaction system? (2 pts)

      a.) Increase the concentration of sucrose three-fold, to about 180 mM.
      b.) Lower the temperature of the reaction system.
      c.) Add an allosteric activator that improves the affinity of this enzyme for its substrates.
      d.) Increase the concentration of water in the system.
      e.) Raise the concentration of the sucrase in the system.

      Answer: _____

14.) The blank plot below has a Y axis of increasing free energy, and an X axis of progress forward for the following reaction: $B + C \rightleftharpoons D + E$
Assume that this reaction is endergonic in terms of its net change in free energy. In the plot indicate for this reaction the relative energy position of reactants and products, and that of the intermediate state. (3 pts)

Relative Free Energy (Joules per mole)

Reactants   Intermediates   Products

Stage of the Reacton

5
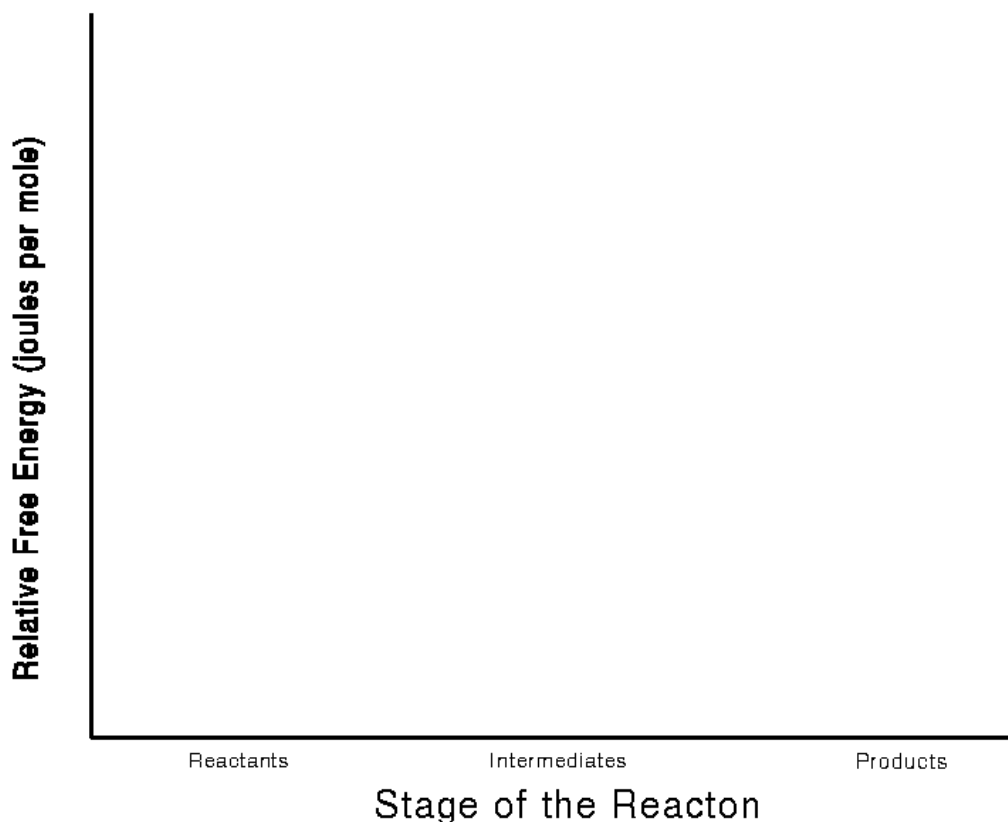
15.) One way in which many cells often keep a reaction far from equilibrium is to: (1 pt)

       a.) produce more enzymes that catalyze the reaction.
       b.) move the products out of a compartment so that they do not accumulate there.
       c.) covalently modify the enzyme that catalyzes this reaction to turn it on.
       d.) use energy derived from catabolic reactions to stop enzymes from catalyzing
            the reaction.
       e.) degrade the enzymes that typically catalyze this reaction.

          Answer: _____

16.) Answer the following questions. (6 pts)

a.) One compartment in an animal cell that typically lacks functional DNA is:	
b.) Two specific compartments in a plant cell where functional ribosomes are often found are:	
c.) To get a membrane protein into the plasma membrane of an animal cell it will likely first have been in the membranes of which two other organelles?	
d.) Stable microtubules are typically found in what one structure of a eukaryotic cell?	

17.) Which one of the following typically has the highest surface area to volume ratio in a eukaryotic cell? (2 pts)

       a.) The membrane of the smooth endoplasmic
            reticulum.
       b.) The outer mitochondrial membrane.
       c.) The membrane of a lysosome.
       d.) The membrane of a peroxisome.
       e.) The inner nuclear envelope membrane.

          Answer: _____
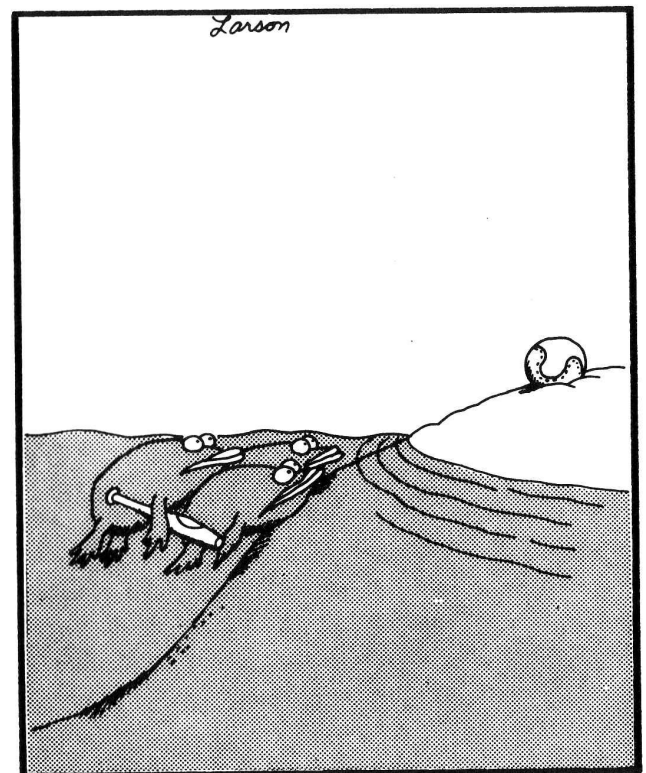
Great moments in evolution

6

18.) Compared to the size of a typical human skin cell, a typical prokaryote, such as *E. coli*, is: (1 pt)

      a.) About ten fold larger in diameter.
      b.) Two to three times larger in diameter.
      c.) Roughly the same diameter.
      d.) About thirty fold smaller in diameter.
      e.) About a thousand fold smaller in diameter.

      Answer: _____

19.) Which is NOT a correct difference between gap junctions and plasmodesmata? (2 pts)

      a.) Gap junctions form between animal cells while plasmodesmata are between plant cells.
      b.) Gap junctions are pores made by integral membrane proteins, while plasmodesmata do not have pores extending across membranes.
      c.) Gap junctions allow cells to share small cytosolic items, while plasmodesmata also have some items in the membrane pass from cell-to-cell.
      d.) New gap junctions can form much more rapidly than can new plasmodesmata.
      e.) Gap junctions allow organelles to pass from cell-to-cell, while plasmodesmata allow just the endoplasmic reticulum to pass from cell-to-cell.

      Answer: _____

20.) Which choice BEST completes the following? The osmotic uptake of water by a cell and the diffusion of CO_2 into the same cell: (2 pts)

      a.) are similar processes because both water and CO_2 can move by passive diffusion across the plasma membrane.
      b.) differ because water is taken up by active transport and CO_2 requires carrier proteins to cross the membrane.
      c.) compete with each other for the energy derived from ATP hydrolysis to power their entry into the cell.
      d.) rarely occur simultaneously.
      e.) both depend on the lower osmolarity of the cytoplasm relative to the medium surrounding the cell.

      Answer: _____

21.) Balancing water potential inside versus outside of a cell while still maintaining cellular contents that are distinct from the environment is a major function cells perform at their cell membrane. For **two** (just 2!) of the following situations describe a typical mechanism used, and identify the type(s) of energy gradients that are being manipulated, in order to achieve water balance by that cell in its surroundings. (6 pts)

a.) Paramecium, a freshwater protist.

b.) A fungal cell in freshwater.

c.) A white blood cell exposed to blood serum with a sodium ion concentration higher than that of the cell's cytosol.

22.) Use the appropriate letters to indicate the typical location(s) in a eukaryotic cell of each of the following items. (Note some items may occur in more than one location, and so may need more than one letter for credit.) (7 pts)

C	Cytosol	M	Mitochondrial Matrix
I	Inner Mitochondrial Membrane	S	Inter-membrane Space

_____ Respiratory electron transport chain.   _____ Fermentation.
_____ Pyruvate.   _____ The Citric Acid Cycle.
_____ ATP synthase.   _____ Glycolysis.
_____ An acidic pH and relatively positive electrical potential.

23.) In terms of just the carbons present indicate (by writing yes or no) whether or not each of the following reactions are part of redox reactions or not. (Note: These reactions are not balanced.) (3 pts)

a. _____

$$
\begin{array}{c}
\text{O}=\text{C}-\text{H} \\
\text{H}-\text{C}-\text{OH} \\
\text{H}-\text{C}-\text{O}-\text{P} \\
\text{H}
\end{array}
\longrightarrow
\begin{array}{c}
\text{H} \\
\text{H}-\text{C}-\text{O}-\text{P} \\
\text{C}=\text{O} \\
\text{H}-\text{C}-\text{OH} \\
\text{H}
\end{array}
$$

b. _____

$$
\begin{array}{c}
\text{O}=\text{C}-\text{O}^- \\
\text{C}=\text{O} \\
\text{H}-\text{C}-\text{H} \\
\text{H}
\end{array}
\longrightarrow
\begin{array}{c}
\text{O}=\text{C}=\text{O} \\
+ \\
\text{H} \\
\text{H}-\text{C}-\text{OH} \\
\text{H}-\text{C}-\text{H} \\
\text{H}
\end{array}
$$

c. _____

$$
\begin{array}{c}
\text{O}=\text{C}-\text{H} \\
\text{H}-\text{C}-\text{OH} \\
\text{H}-\text{C}-\text{O}-\text{P} \\
\text{H}
\end{array}
\longrightarrow
\begin{array}{c}
\text{O}=\text{C}-\text{O}\sim\text{P} \\
\text{H}-\text{C}-\text{OH} \\
\text{H}-\text{C}-\text{O}-\text{P} \\
\text{H}
\end{array}
$$
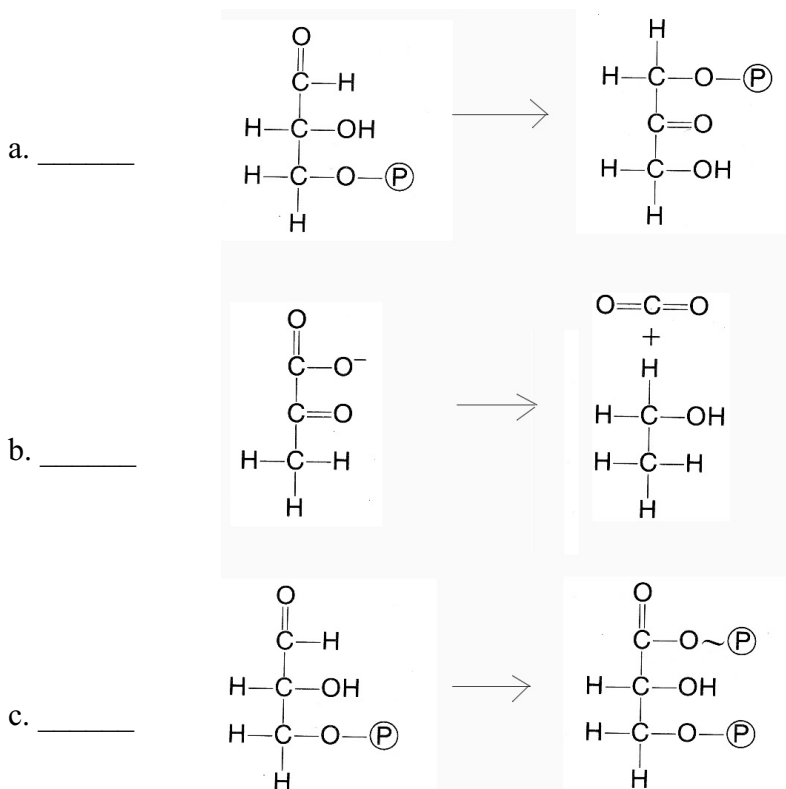
24.) Which statement is LEAST consistent with substrate-level phosphorylation? (1 pt)

a.) There are reactions with a larger free energy change than that of ATP formation.
b.) ADP will often be given a phosphate group from an organic molecule.
c.) Not all covalent bonds require the same amount of energy to break.
d.) To power this type of phosophorylation it must be coupled to the reduction of oxygen gas.
e.) There are many molecules other than ADP that can have a phosphate group.

Answer: _____

25.) (4 pts) If one pyruvate enters the mitochondria how many ATPs would normally be made if oxidative respiration is taken to completion?

_____

Other than ATP, what are two other products that would normally be made?

_____   _____

26.) Draw a cross section of a mitochondrion and a chloroplast showing all the typical membranes and soluble compartments. In your drawings note the locations of the following items by labeling them with the indicated letter: a.) The membrane(s) across which a proton motive force is created. b.) The compartment(s) in which ATP is made by chemiosmotic processes. c.) The compartment(s) in which oxygen gas is either created or consumed. d.) The membrane(s) that is(are) most recently derived from a prokaryote's plasma membrane. (8 pts)
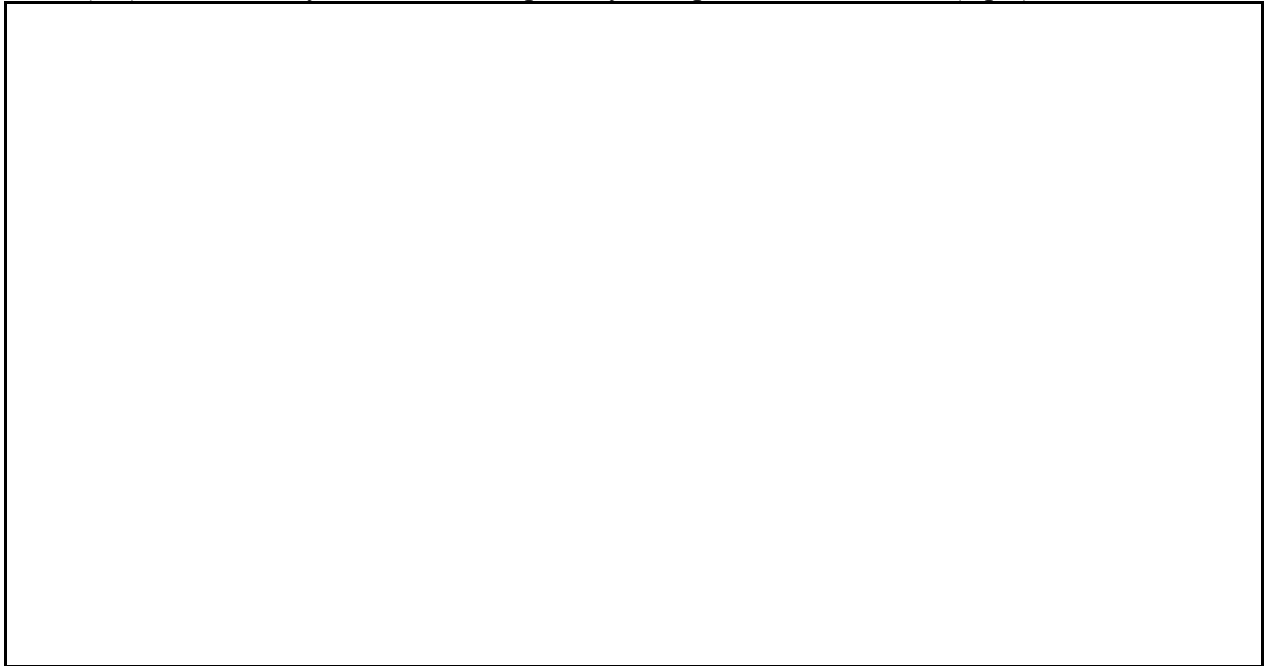
27.) Which one of these molecules typically serves as a direct electron donor to the electron transport chain of in most mitochondria? (1 pt)

      a.) NAD^+.
      b.) Water.
      c.) O_2.
      d.) $FADH_2$.
      e.) Pyruvate.

          Answer: _____

28.) Indicate for each of the following: (4 pts)

The molecule that supplies the electrons used in photosynthesis: _____

The ultimate source of energy used to power the process of photosynthesis:

_____

The original state of carbon brought into photosynthesis: _____

The immediate source of energy that is coupled by the chloroplast ATP synthase to power the formation of ATP:

_____

29.) For **each** of the two processes given below state **one** (1!) net substrate and **one** net product of each process, and indicate the specific location in a plant cell where the process is carried out. (6 pts)

Process	Substrate it uses.	Product it creates.	Specific location.
Light reactions of photosynthesis:			
Calvin cycle:			
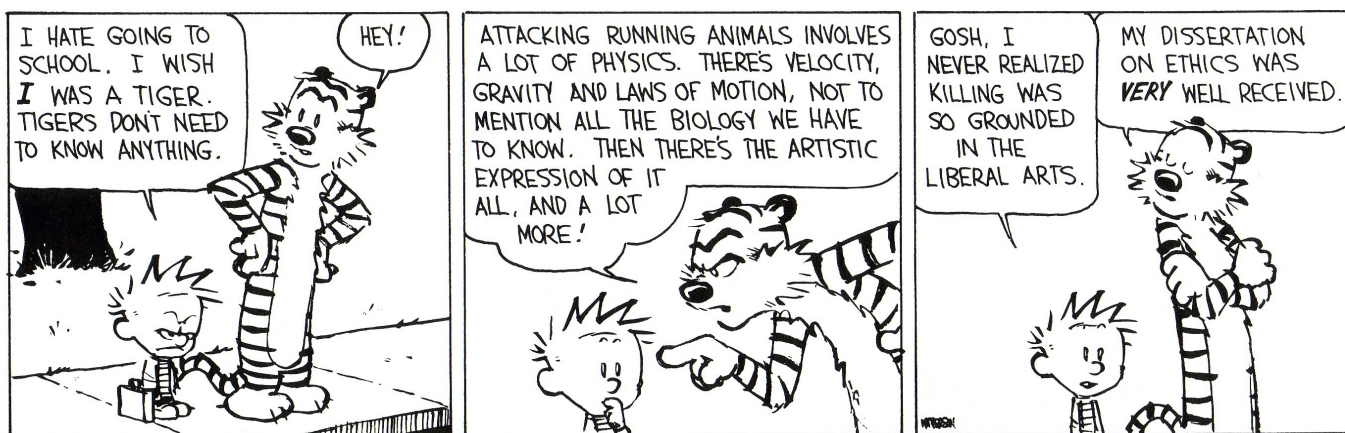
30.) Which one of the following will most likely be needed to turn OFF a phosphorylation cascade in a cell's cytosol after it had been activated? (1 pt)

   a.) The production of new proteins by the expression of genes which have just been turned on.
   b.) The hydrolysis of GTP by an activated G-protein.
   c.) The removal of Ca^{+2} out of the cytosol by Ca^{+2}-pumping ATPases.
   d.) The degradation of inositol-triphosphate (IP_3) by an enzyme.
   e.) The action of specific protein phosphatases.

   Answer: _____

31.) Describe a major difference between a G-protein coupled receptor system compared to a tyrosine-kinase receptor system in terms of how each manages to activate components of cellular transduction systems. (6 pts)

12
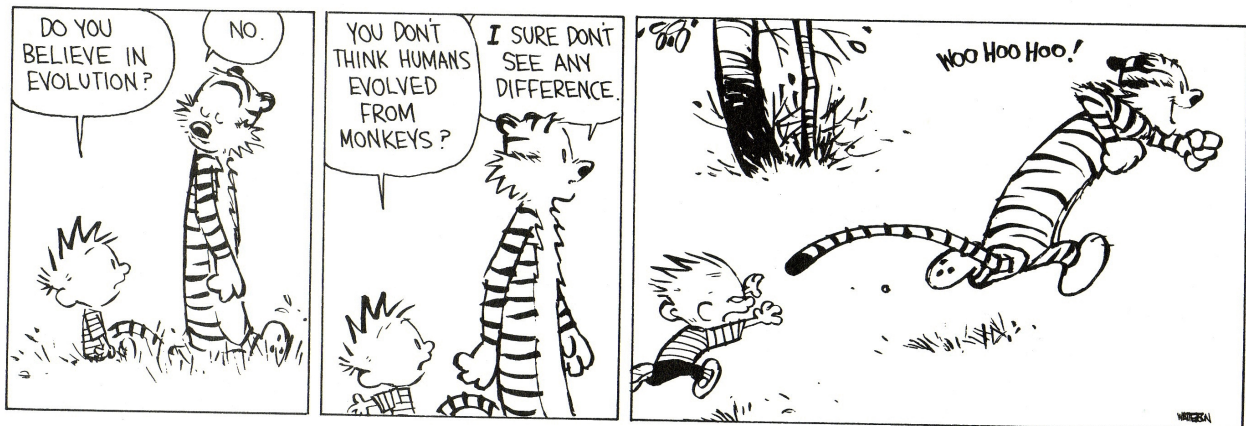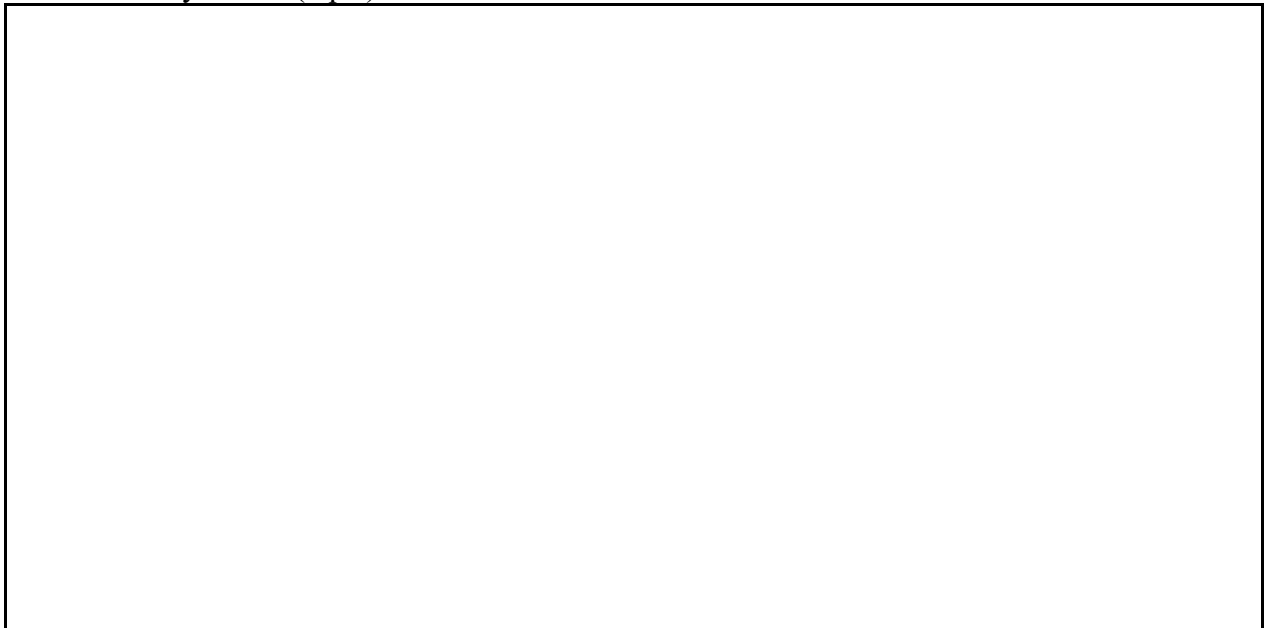
32.) A receptor that is a ligand-gated ion channel when activated typically allows one type of ion to pass through itself in one direction. Answer the following questions about it. (5 pts)

a.) Is the movement of these ions an **active** or a **passive** process? (circle your answer)

b.) Identify two items which activation of this receptor alters that could then be used by the cell in subsequent transduction of information.

c.) Describe the key thing that must be done to reset this receptor so that it can be used again in signal reception.
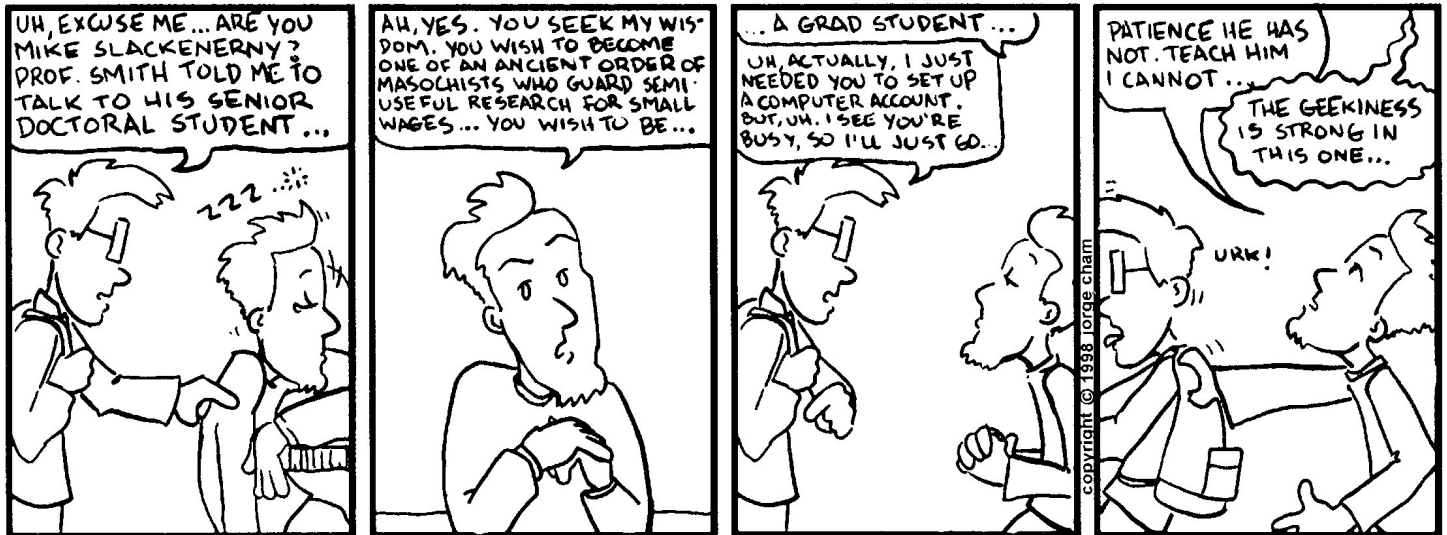
Optional Bonus Question. Any points earned in an answer to this question will be substituted for up to five missed points out of the seventy that this exam is worth. (Please limit your answer to this side of this page.)

Choose two major classes of organic molecules to compare in terms of their abilities to carry out functions necessary for life. For each class note a major function it carries out in life and describe the function's importance in each case. Also relate that class of molecule's ability to interact with other molecules to its ability to carry out the function you identify for it. Do these two classes differ in the range of functions they carry out? If so, then what features are in one class of molecules that causes it to be able to carry out a broader range of functions than the other class?

BIO 107   2010
Exam #1   Proposed Answers:

        Below are proposed answers for the questions in this exam.

        Be aware that for many of these questions other answers exist that may also be acceptable either for full or partial credit.

_____

1.) Inductive reasoning produces from a body of knowledge a summary that attempts to take all that knowledge into account. Deductive reasoning uses such a summary and creates a set of expectations, or predictions, of what should be encountered in new situations. Thus induction requires no checking with new information or tests, while deduction invites testing.

2.) Marked should be the second, fourth, and last item in this list.

3.)
        Null hypothesis: There will be no significant difference between the blood pressures observed in the mice treated with the drug compared to those mice not treated with the drug.
        Alternative hypothesis: There will be a significant difference between the blood pressures observed in the mice treated with the drug compared to those mice not treated with the drug.

4.)
        Structure: Membranes.
        Its Function: To control what remains in the life form relative to the outside.

5.) C.
6.) D.

7.) Reading down the list the rankings should be: 1, 3, 4, 2.

8.) D.
9.)   a.) 5
         b.) hydroxyl, ketone, methyl
         c.) polar covalent bond: C-N
            non-polar covalent bond: C-C
                (And circle and example of each of these two bonds in the figure.)
10.) D.
11.) ..... membranes...... hydrophobic .....
12.) C.
13.) E.

1

14.)

   The reactants (B + C) should be relatively lower than the products (D + E).

   The intermediates should be at a higher energy level than either of the reactants or products to indicate the presence of a large activation energy barrier.

15.) B.
16.)   a.) cytosol
        b.) cytosol, stroma
        c.) golgi, endoplasmic reticulum
        d.) cilia
17.) A.
18.) D.
19.) E.
20.) A.

21.) (Only two answers were needed here...)

        a.) They use a contractile vacuole to create energy in a pressure gradient to force out the excess water. Thus they keep their water content constant, even though there is an energy gradient pulling the water in to themselves.

        b.) The fungi cell has a cell wall. This allows the cell to have a higher pressure (i.e. turgor) than the outside. The energy in this pressure gradient is used to balance the oppositely aligned energy gradient that is based on osmotic differences. Thus these two energies combine to give water in the cell the same energy status as the outside water.

        c.) The blood cell's plasma membrane has transport systems in it that move in selected ions, other than sodium, so that the osmotic content of the cell is the same as the blood serum even though the composition differs. With the same pressure and same osmolarities, this produces a balance of energy for the inside and outside water for this cell.

22.) The most likely answers would be:
        I   Repiratory electron transport chain.
        C   Pyruvate
        I   ATP synthase
        S   An acidic pH and relatively positive electrical potential
        C   Fermentation
        M   The citric acid cycle
        C   Glycolysis

23.)   a.) No
        b.) Yes
        c.) Yes

24.) D.

25.)
        15 ATP
        CO_2, H_2O
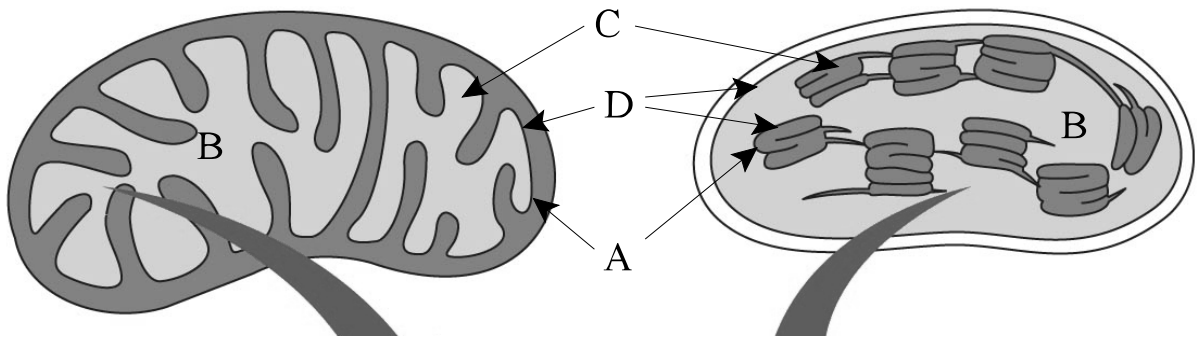
26.) (See fig. 10.16, on pg. 196 of our text.)

27.) D.

28.) H_2O
      Light
      CO_2
      An electrochemical proton gradient.

29.) (There are other substrates and produces that are acceptable here...)

Process	Substrate it uses.	Product it creates.	Specific location.
Light reactions of photosynthesis:	H_2O	O_2	Thylakoid membranes
Calvin cycle:	ATP	ADP	Stroma

30.) E.

31.)

One difference is in whether or not the receptor uses covalent bond modification to activate transduction systems.

The tyrosine kinase receptor will first form covalent bonds to phosphorylate themselves, and then will make phosphoester bonds to transfer these phosphate groups to its targets.

The G protein coupled receptor does not make use of covalent bond modification. It induces its associated G protein to bind GTP, and then releases the activated G protein so that it can interact with other proteins and alter their activity.

32.) a.) passive

b.)   The concentration of the ion in the cytosol.
        The electrical potential across the plasma membrane.

c.) The ligand must be released from this receptor's binding site.

BIO 107   Exam #2   Summer 2010
Thursday, July 1   Name: _____
        Points earned: _____ = _____ for course.
                                    70
Please keep your answers in the spaces provided as answers outside of there will not be read!
Good luck!

1.) If you wish to tell if an organelle in a protist was the result of secondary endosymbiosis rather than primary endosymbiosis, then what distinctive feature would you look for in this protist that would let you make a decision? (2 pts)

2.) Which one of the following is NOT a characteristic that is shared by some protists and humans? (1 pt)

        a.) Possession of a nucleus.
        b.) The use of microtubules during mitosis.
        c.) They have, on at least some cells, flagella that are bounded by the plasma membrane.
        d.) Many of them have the ability to carry out photosynthesis.
        e.) The ability to carry out aerobic cellular respiration.

                Answer: _____

3.) If a protist lives in freshwater, but lacks a cell wall, how can it avoid swelling and bursting due to osmosis? Identify a likely mechanism and indicate one advantage and one disadvantage of using this mechanism relative to having a cell wall. (3 pts)

1

4.) Which item below is NOT adaptive for reducing the loss of heat from an endothermic animal out to a cold environment? (2 pts)

     a.) Vasoconstriction of arterioles leading to capillaries just under the skin.
     b.) Having a thick layer of fat associated with the skin.
     c.) Using a counter current system to retain heat in the body core.
     d.) Creating more internal heat in brown fat areas of the body.
     e.) Growing a thick layer of hair or fur.

         Answer: _____

5.) Select two (Just 2! i.e. 1+1!) of the items given below. For each selected subcellular item describe an example of a problem in a multicellular organism that uses that item to overcome the problem, and describe how it is used to achieve that result. (4 pts)

Microfilaments:
Surface adhesion proteins:
Cilia:
Exocytosis:

6.) Identify an example of a counter-current system, describe what effect it has, and note how this is adaptive to the organism. (3 pts)

7.) The villi of the small intestine do all of the following EXCEPT for: (2 pts)

   a.) acting as a site of digestion of food.
   b.) transfer hydrophilic items to blood in the capillary beds within the villi.
   c.) increasing the surface area available for absorption of food.
   d.) help to push the food through the intestine.
   e.) pass hydrophobic items to lymphatic lacteals within the villi.

   Answer: _____

8.) A distinct advantage of extracellular digestion over intracellular digestion is that: (2 pts)

   a.) polymers are hydrolyzed to monomers by digestive enzymes.
   b.) there is smaller surface area for absorption of digested items.
   c.) it makes it possible for items that are larger than the organism's cells to be used as a food source.
   d.) all types of macromolecules can be digested instead of just glucose.
   e.) there is no need for a transport system to move the products of extracellular digestion to all the cells of the body.

   Answer: _____

Larson
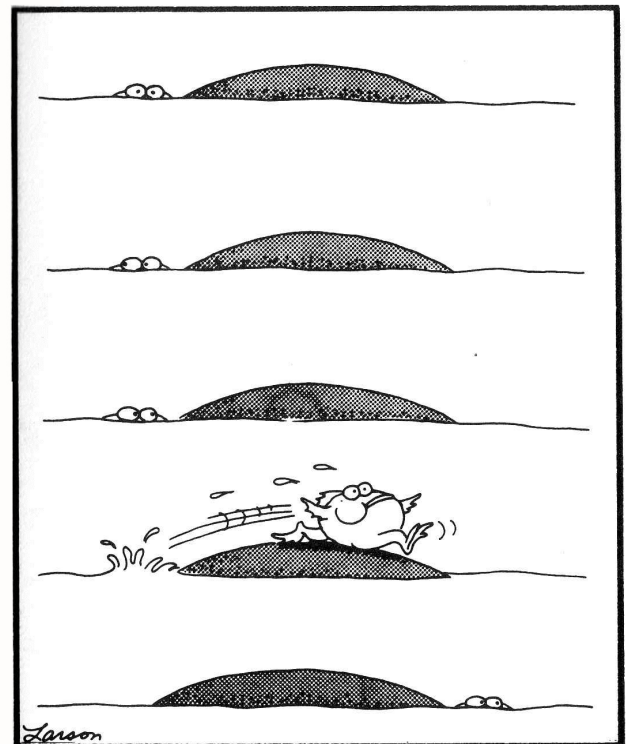
Another great moment in evolution

3

9.) Which choice best accounts for why cells of our body secrete a pancreatic amylase into the small intestine when other cells have already secreted a salivary amylase into our mouth? (2 pts)

a.) The salivary amylase digests starch, while the pancreatic amylase digests glycogen.
b.) The acidic pH and presence of pepsin will denature and degrade salivary amylase in the stomach, so an additional amylase is needed in the small intestine.
c.) Pancreatic amylase has a broader pH optimum than salivary amylase.
d.) Salivary amylase is secreted by salivary glands and never leaves the mouth.
e.) Unlike salivary amylase, pancreatic amylase is unable to digest parts of cells lining the digestive tract.

Answer: _____

10.) An animal species lives on a food source that is extremely resistant to digestion by the enzymes the animal can produce. Describe one way this animal's digestive system could be altered and how this alteration could enhance its ability to get something out of this food. (2 pts)

11.) Beginning in a vein coming from the kidney (the renal vein) and ending in the systematic aorta, if you order the following structures of the human circulatory system in the proper sequence in which they would be encountered, which structure would be THIRD? (2 pts)

a.) Posterior vena cava.
b.) Left ventricle.
c.) Pulmonary artery.
d.) Pulmonary vein.
e.) Right atrium.

Answer: _____
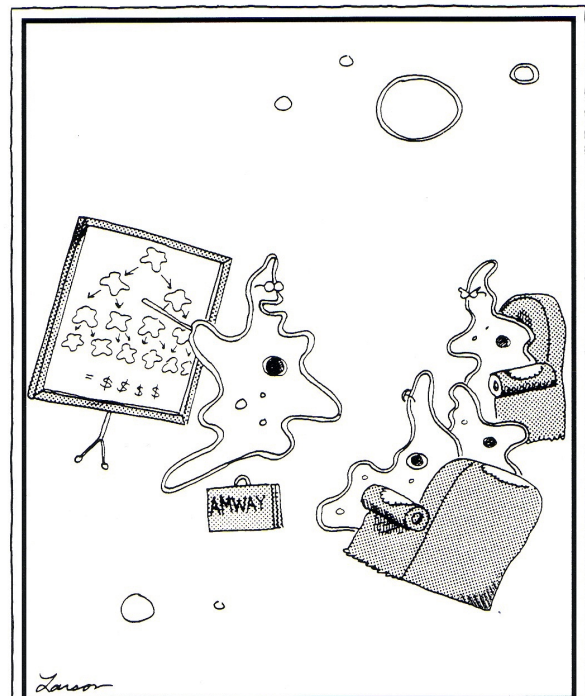
"And, as amoebas, you'll have no problems recruiting other sales reps … just keep dividing and selling, dividing and selling."

4

12.) Below are five statements concerning the mammalian circulatory system. Each statement consists of a description of a characteristic of the circulatory system and an outcome of that characteristic. Which of the following INCORRECTLY states a characteristic, or mismatches it with its outcome? (2 pts)

   a.) The volume per unit length of the circulatory system is largest in the capillary beds, which means that the blood in these beds has the lowest flow rate in the circulatory system.
   b.) The blood pressure is higher at the arteriole end of the capillaries than at the venous end but the solute concentration of the blood is nearly constant through the capillaries, which causes there to be a loss of water from the blood at the arteriole end and recovery of water into the blood at the venous end of the capillary bed.
   c.) There are precapillary sphincter muscles present at various points leading to most capillary beds, this permits the control of blood flow to individual systemic capillary beds.
   d.) The highest pressures in the circulatory system occur in the arteries, thus the arteries have the thickest walls (compared to the veins and capillaries).
   e.) Once stimulated by signals initiated by the AV node, the SA node sends signals to the ventricles, this stimulates the contraction of the right ventricle before the left ventricle so that blood can return to the systemic circulatory loop from the pulmonary loop.

   Answer: _____

13.) Fill in the following blanks with the most appropriate words or phrases. (4 pts)
At birth, the closing of the *foramen ovale* removes a connection between the

_____ and the _____ of the human heart. At the

same time the closing of the *ductous arteriosus* removes a connection between the

_____ and the _____.

14.) Which choice would be the best generalization to complete the following (i.e which would have the least exceptions)? Across all animal species that have a circulatory system, whether they have an open or a closed circulatory system, they will all have: (1 pt)

   a.) capillary beds associated with gills.
   b.) distinct blood and lymphatic fluids.
   c.) a rapid flow of blood through capillary beds to enhance the exchange of items.
   d.) a major role of the circulatory system in carrying out gas exchange in that species.
   e.) pressure in an artery higher than the pressure in a vein.

          Answer: _____

15.) Which would have the better (i.e. stronger) affinity for oxygen adult hemoglobin or fetal hemoglobin?

_____

Also draw the hemoglobin oxygen saturation curves for adult (A) and fetal (F) hemoglobin in the figure below (clearly label each curve with the appropriate letter). (6 pts)
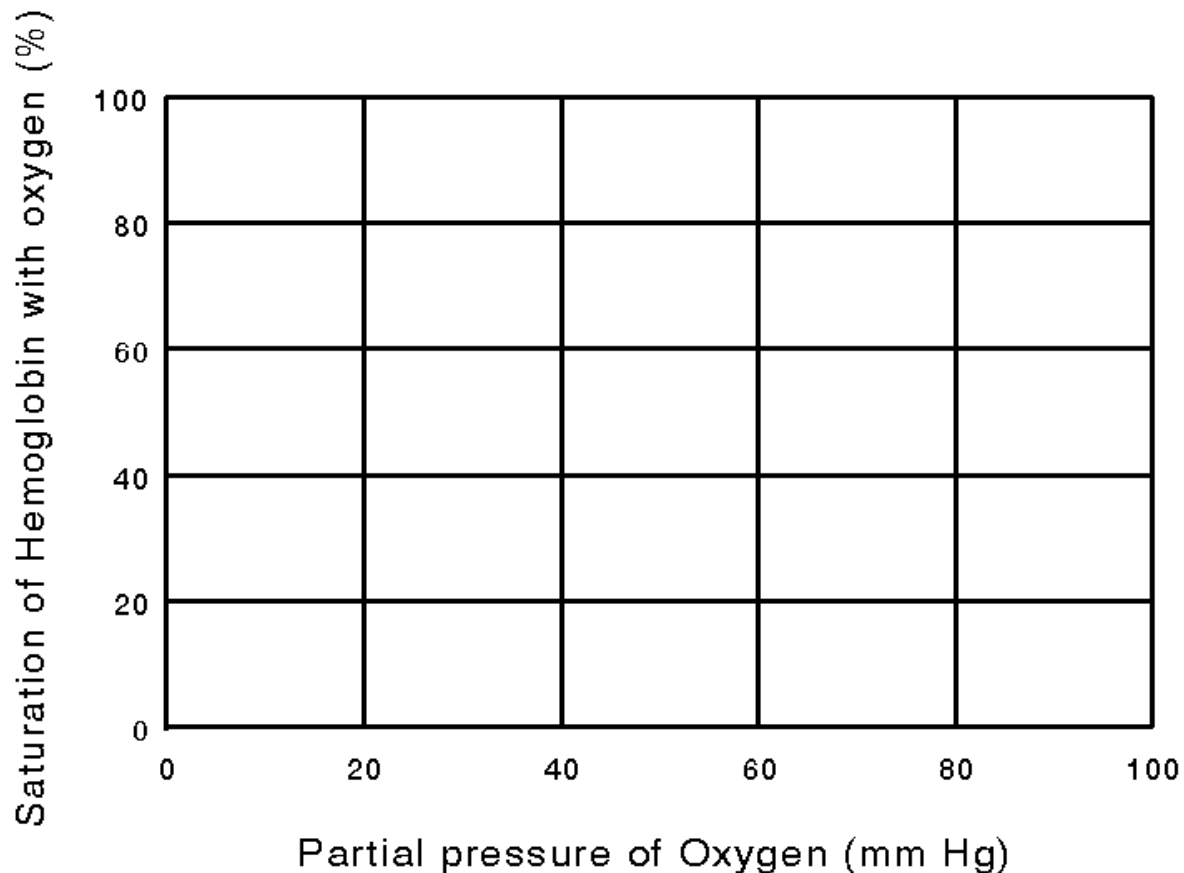
16.) In the pathway of oxygen gas exchange in humans much of the movement of the gas is driven solely by diffusion. Which of the following is **NOT** solely driven by diffusion? (2 pts)

    a.) Movement from the alveoli into the blood in a neighboring capillary bed.
    b.) Passing from the intercellular fluid of the body up to the cells themselves.
    c.) Moving through the water covering the epithelial cells of the lungs.
    d.) Passing across the plasma membranes that surround cells of the body.
    e.) Traveling through a capillary bed from its arteriole end to its venule end.

        Answer: _____

17.) In countercurrent exchange: (2 pts)

    a.) energy from the hydrolysis of ATP is used to enhance the diffusion of oxygen.
    b.) opposite orientated flows allows the transfer of some item(s) for the entire length of contact.
    c.) oxygenated blood is carried out to the body and deoxygenated blood returns from the body.
    d.) excess heat is sent to the body extremities so that it can be radiated out to the environment.
    e.) the capillaries in the gills pick up more oxygen than do capillaries in the body tissues.

        Answer: _____

18.) Which one of the following choices does NOT describe a problem that must be surmounted to form a root nodule? (1 pt)

    a.) The plant must be able to take up enough nitrate and ammonia from the soil.
    b.) The plant must be able to form a symbiotic association with the right type of bacterium.
    c.) The plant must make new vascular connections to the nodule tissue so that it can be supplied with sugars.
    d.) The plant must exchange the proper signals with the bacterium before the symbiosis can be established.
    e.) The plant must induce a correct combination of gene expression in the bacteria.

        Answer: _____

19.) Inorganic mineral nutrients are used for a wide range of processes in plants. Which of the following is NOT a correct statement concerning how plants use mineral nutrients? (2 pts)

    a.) They may be used as cofactors in enzyme catalyzed reactions.
    b.) They may be involved in essential steps of the light reactions of photosynthesis.
    c.) Those minerals taken from the soil end up accounting for most of the mass of organic matter the plant will make.
    d.) Some of these minerals can act as a second messenger in signal-transduction systems.
    e.) Some of these might be accumulated or expelled as a cell attempts to regulate the concentration of its osmotically active substances.

    Answer: _____

20.) Which pair of terms best completes the following statement? Mycorrhizae are most likely to supply plants with _____, and in turn are likely to receive _____ from the plant. (1 pt)

    a.) water; minerals
    b.) ATP; hormones
    c.) minerals; sucrose
    d.) organic matter; water
    e.) good anchorage; assistance in dispersal

    Answer: _____

21.) Which is **NOT** a means that a plant is likely to use to try to gain more access to soil nutrients? (2 pts)

    a.) secrete organic compounds that chelate selected ions and make them more mobile.
    b.) secrete acids to alter the soil pH.
    c.) secrete organic compounds to feed soil microbes which then mobilize nutrients from the soil.
    d.) enter into a symbiotic interaction with soil fungi.
    e.) export oxygen to the soil to oxidize soil nutrients so that they are more mobile.

    Answer: _____
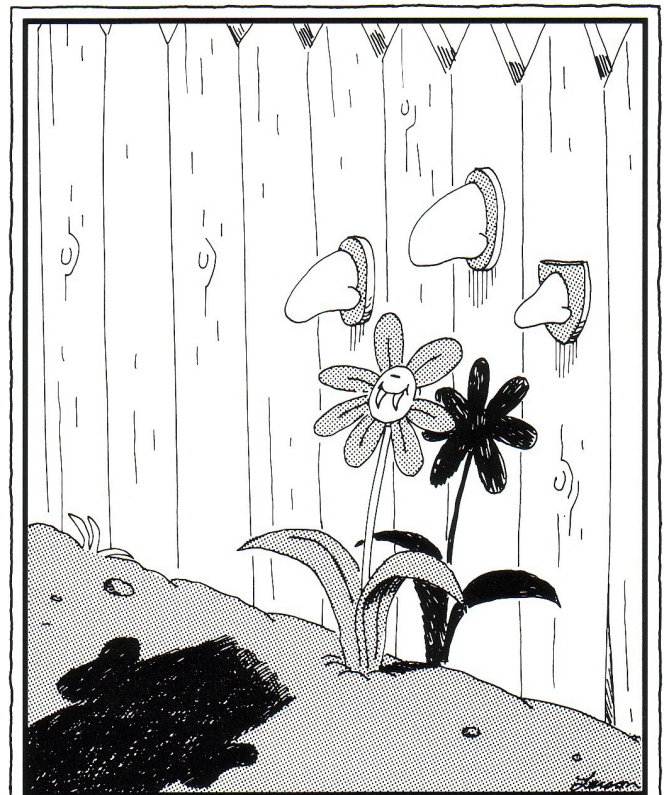
22.) Which of the following processes, all carried out in human nephrons, is typically the LEAST selective? (1 pt)

    a.) Reabsorption of glucose from the filtrate.
    b.) Production of the filtrate at the Bowman's capsule.
    c.) Active transport of sodium ions by part of the loop of Henle.
    d.) The role of aquaporins in cells of the collecting duct in osmosis.
    e.) Cotransport of sodium ions with the amino acid alanine.

    Answer: _____

23.) In the figure at right indicate the location of each of the following items by drawing a line to it and labeling the end of each line with the appropriate letter. (8 pts)

    a.) Collecting duct.
    b.) A small vein.
    c.) Descending Loop of Henle.
    d.) Glomerulus.
    e.) Area where filtration occurs.
    f.) One area where reabsorption occurs.
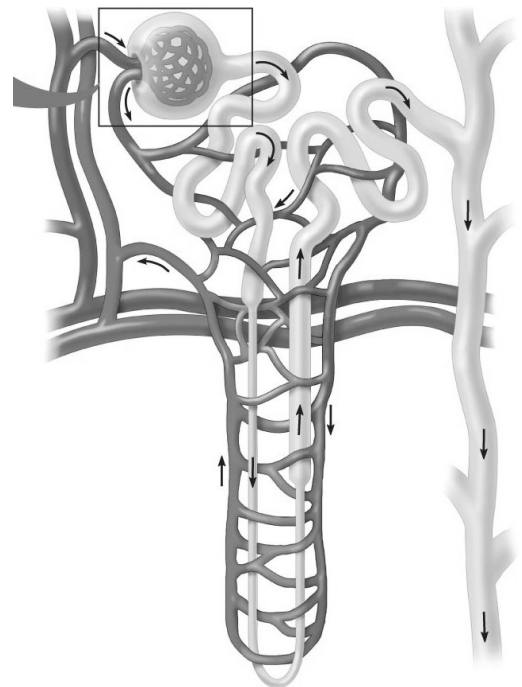
This structure is collectively called a

    _____.

This structure is typically found
       in which organ?

    _____

24.) In a sarcomere the type of bond that is formed between actin and myosin is: (1 pt)

    a.) a polar covalent bond.
    b.) a non-polar covalent bond.
    c.) a hydrogen bond.
    d.) an ionic bond.
    e.) based on van der Waals interactions.
    f.) an emotional bond.

    Answer: _____

25.) The role of calcium ions during muscle contraction is to: (1 pt)

    a.) act as a cofactor in breaking bonds between actin and myosin.
    b.) depolarize the plasma membrane of the muscle cell as part of its action potential.
    c.) be accumulated in the sarcoplasmic reticulum of the myofibril.
    d.) be bound by the troponin that is associated with the thin filaments of the sarcomeres.
    e.) strengthen the Z-lines so that they can withstand the forces pulling on them.

        Answer: _____

26.) Answer the following questions about motor proteins. (5 pts)
a.) What are two types of motor proteins?
        For each identify the type of cytoskeletal structure down which it moves.

Motor protein:	Cytoskeletal structure:

b.) Motor proteins typically have what specific enzymatic activity (i.e. they catalyze what reaction)?

_____

27.) If a plant makes many offspring, but fails to achieve dispersion of its offspring, what is one problem that is likely to occur? (2 pts)
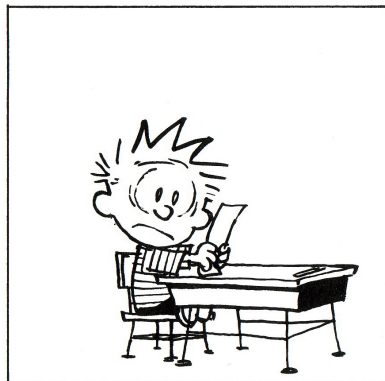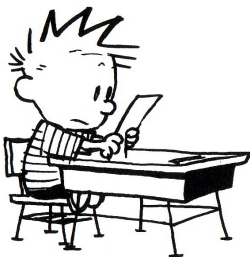
28.) Match the one most appropriate letter of each of the following means of dispersal of seeds used by some plants with each of the sets of traits given below. (6 pts)

    a.) Wind Dispersal.
    b.) Dispersal on the water.
    c.) Dispersal by biotic means involving being on the outside of an animal.
    d.) Dispersal by biotic means involving being on the inside of an animal.

    _____ A tough hard inner fruit coat surrounds a seed, but the outer fruit coat is soft and full of sugars.
    _____ The fruit coat has a high surface area to volume ratio, and is dry and light.
    _____ The fruit coat may be waterproof, round in shape, and filled with pockets of air.
    _____ The soft outer fruit coat is succulent and surrounds an inner core with toxic digestible seeds.
    _____ A dry set of branches with many fruits on it breaks off and is rounded in shape so that it rolls well.
    _____ Part of the fruit coat forms a sharp spine that can pierce the skin if stepped on.

29.) Consider the regulation of calcium concentration in the blood and its uses by cells. (6 pts)
a.) If a mutation occurs in the gene that codes for a PTH (parathyroid hormone) receptor in cells in the lining of the nephron of the kidney such that the receptor does not function, what effect is that likely to have on what function of these cells?

b.) Identify and describe the hormone whose action is antagonistic to PTH. Note which endocrine gland secretes it, the conditions that stimulate that secretion, and describe how the effects of this hormone are consistent with its being antagonistic in effect to PTH.

30.) A portal vein carries blood from the hypothalamus directly to the: (1 pt)

    a.) liver.
    b.) anterior pituitary.
    c.) thalamus.
    d.) posterior pituitary.
    e.) kidney.

       Answer: _____

31.) Describe a major difference between the items given in two (just 2!) of the pairs listed below. (4 pts)

Hormones and pheromones:

Hormones and secondary messengers:

Hormones and growth factors:

Hormones and neurotransmitters:

32.) In mammals entry into labor for the initiation of the birth process is typically triggered by: (1 pt)
    a.) human chorionic gonadotropin (HCG) made by the fetus.
    b.) a rise in the concentration of oxytocin.
    c.) a drop in the concentration of progesterone.
    d.) a sudden rise in the concentration of luteinizing hormone (LH).
    e.) a loss of response of the uterus to estrogens.

       Answer: _____

33.) Which of the following lists the mammalian male genital structures in the CORRECT order in which sperm normally passes through them? (2 pts)

    a.) epididymis - seminiferous tubules - vas deferens - seminal vesicle - penis
    b.) seminiferous tubule - epididymis - vas deferens - urethra - penis
    c.) epididymis - seminiferous tubules - urethra - vas deferens - penis
    d.) vas deferens - urethra - epididymis - penis - seminiferous tubules
    e.) epididymis - urethra - vas deferens - penis

       Answer: _____

34.) Below is a figure showing the human menstrual cycle with days and its phases indicated on the X axis. Write the letter for each event in the space immediately below the figure for the time when each event occurs relative to the menstrual cycle, and to each other. (5 pts)

    a.) The point of highest concentration of LH.
    b.) The point of highest concentration of progesterone.
    c.) When the follicle will have few or no receptors for LH.
    d.) The time when ovulation will occur.
    e.) A time when there will be an active *corpus luteum*.

Bleeding and sloughing of uterine lining

←Menses→ ←— Proliferation —→ ←— Secretory phase —→

0   5   10   15   20   25
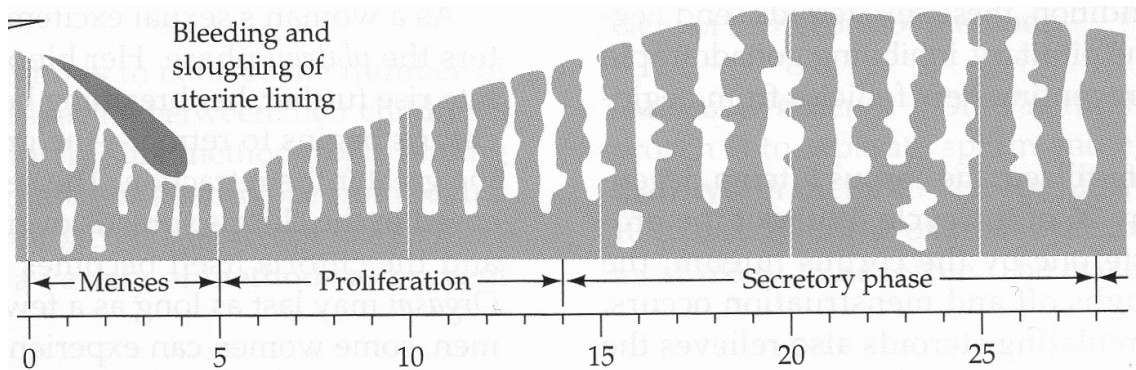
35.) Number the following structures from first (1) to last (5) in terms of which a water molecule in the soil is likely to encounter as it travels into and through a vascular plant. (5 pts)

    _____ Casparian strip.   _____ A root hair.
    _____ Vessel member in xylem tissue.   _____ Cell wall of a mesophyll cell.
    _____ Air inside of a leaf.

36.) Which BEST explains the movement of sucrose from source to sink in a plant? (1 pt)

a.) The evaporation of water from the leaves and active transport of sucrose from the sink.
b.) The creation of higher osmotic pressure in sieve tubes in a source region relative to that in the sink region.
c.) Tension created by the pulling of sucrose out of sieve tubes at source tissue.
d.) Pressure generated by the contraction of sieve tubes powered by ion pumps.
e.) The hydrolysis of starch to form sugars which raise the water potential in the source relative to the sink tissues.

Answer: _____

37.) Which BEST describes a major role of the Casparian strip? (1 pts)

a.) This is a layer of wax secreted by the epidermis of leaves which blocks the loss of water from the leaves.
b.) It is where ions and other desired solutes are pumped from the cortex into the vascular bundle of the root.
c.) It forms a barrier between the apoplastic space of the vascular bundle and the apoplastic space of the root cortex.
d.) This region of the cell wall is very strong and provides structural support to the vascular bundle it surrounds.
e.) This is a selectively permeable barrier in the root through which only desired items are allowed to pass.

Answer: _____
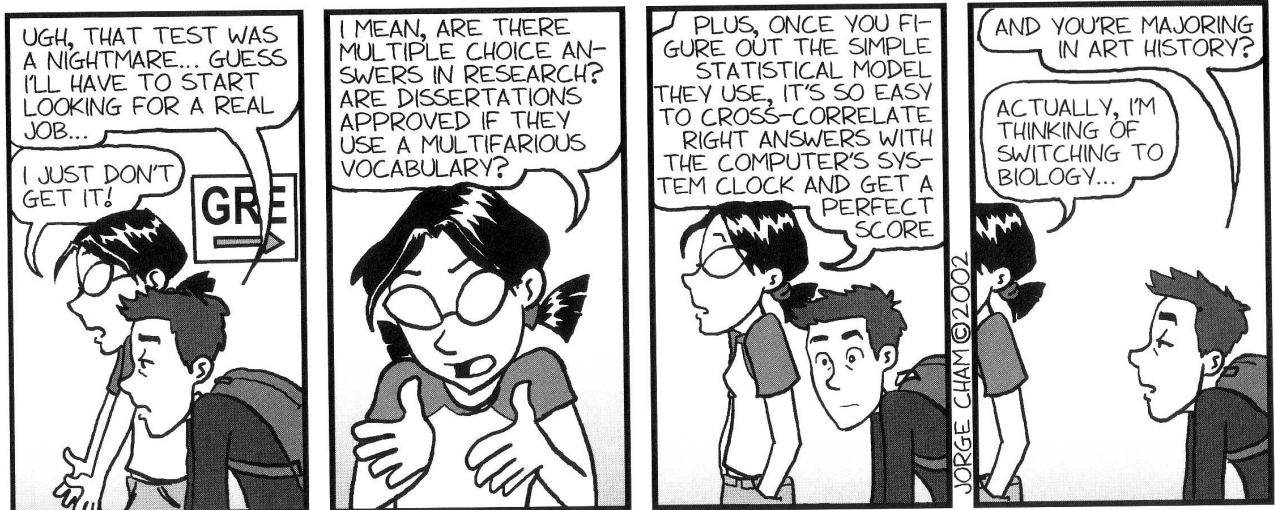
Optional Bonus Question. Any points earned in an answer to this question will be substituted for up to five missed points out of the 70 points that this exam is worth. (Please limit your answer to this side of this page.)

Both vascular plants and large animals are often too large to depend on diffusion alone for the internal exchange of items. Compare the ways that they each actively manipulate components of water potential and how this helps them to achieve internal bulk flow of items through their bodies. For each: Identify and note the role of a key cellular type in the process. Identify an active molecular process involved. Contrast plants and animals in terms of how these molecular and cellular systems result in bulk flow in each case.

BIO 107   2010
Exam #2   Proposed Answers:

        Below are proposed answers for the questions in this exam.

        Be aware that for many of these questions other answers exist that may also be acceptable either for full or partial credit.

1.) Organelles made by primary endosymbiosis tend to have two membranes (the bacterial cell membrane and another from the eukaryote). While an organelle made by secondary endosymbiosis will tend to have more than two bounding membranes. (See fig. 28.2, pg. 577, of our text.)

2.) D.

3.) A cell could use a contractile vacuole. It would reabsorb the desired osmotically actively items across its membrane back into the cell's cytsol, and then pump the excess water out of the cell. This would allow a cell that lacks a cell wall to avoid turgor pressure and so allow it to expose its bare plasma membrane, which might be useful if it wants to do phagocytosis. But reabsorbing the ions and generating the force to constrict the vacuole will cost the cell energy.

4.) D.

5.) (Only two of these needed to be answered.)

 Microfilaments: Myosin pulls on these to generate force needed for constriction of a heart in humans to power the flow of blood, which is necessary for proper circulation.

 Surface adhesion proteins: Cells lining the gastrovascular cavity of hydra use these between their epithelial cells so that the items in the body fluids are distinct from the items in the gastrovascular cavity. This allows the organism to have distinct compartments.

 Cilia: Humans use these in the epithelial cells of our trachea to move dust and mucus up out of our respiratory tract. This allows the large area of our respiratory system to remain open and unobstructed, which is good for continued gas exchange.

 Exocytosis: Human neuron cells communicate to other cells via exocytosis of vesicles that contain neurotransmitters. This allows a type of cell-to-cell communication in the organism.

6.) (See fig. 40.12, pg. 865 of our text. Other examples are possible answers here as well.) In the leg of a duck the flow of the blood in the arteries down the leg is opposite to that of the blood in the veins up the leg. This allows heat from the warm arterial blood to be taken up by the cooler venous blood, and so retains more of the heat in the core of the duck's body and keeps some of that heat from being taken to the extemities and being lost. This saves the duck some of the energy it costs to keep warm, and so is adaptive.

7.) D.

8.) C.

9.) B.

10.) The caecum (i.e. appendix) could be expanded and helpful mutualistic microbes could be encouraged to live in it. If those microbes have the ability to digest the food well, then some of those products of digestion would be available to the host animal.

11.) (See fig. 42.6, pg. 903 of our text.) C.

12.) (See fig. 42.9, pg. 905 of our text.) E.

13.) .... right atrium ...... left atrium....
       ...... systemic aorta ...... pulmonary aorta......

14.) E.

15.) Fetal Hemoglobin
     (See the figure for question #11
     on pg. 929 of our text.)
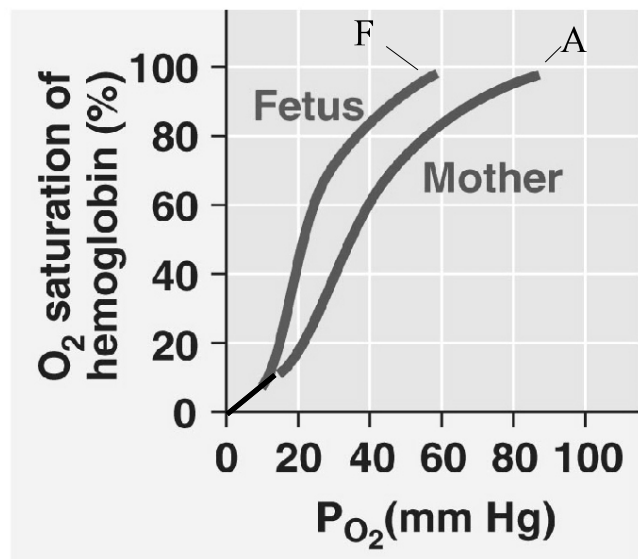
16.) E.

17.) B.

18.) A.

19.) C.

20.) C.

21.) E.

22.) B.

23.) (See fig. 44.14, on pg. 963 of our text.)
     The structure is a: Nephron.
     It is in the: Kidney.
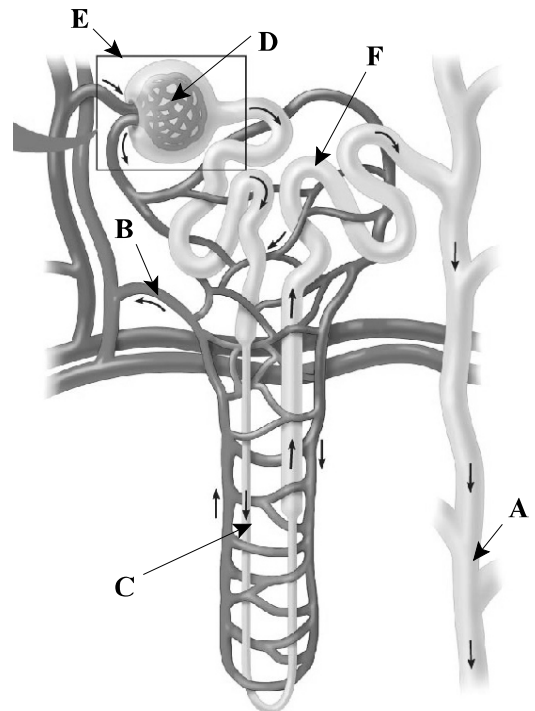
24.) C.

25.) D.

26.)

    a.)
    Myosin - microfilaments
    Dynein - microtubules
    b.)
    ATP hydrolysis.

2

27.) They will all try to live in one spot. This will cause alot of competition for water, soil minerals, and light.

28.) The order down the list should be: D, A, B, C, A, C.

29.) (See pg. 991 of our text.)
  a.) Since they can not detect PTH, the cells will not take up calcium ions from the filtrate when they should. This may deprive the body of some needed calcium.
  b.) Calcitonin is secreted by the thyroid gland when the concentration of blood calcium ions raises above a set point. Calcitonin then stimulates deposition of calcium into new bone and inhibits the uptake of calcium ions by the intestines and the kidneys. Thus where PTH causes the blood calcium ion concentration to raise, calcitonin causes it to fall.

30.) B.

31.) (Only two of these needed to be answered. See fig. 45.2, on pg. 976 of our text.)

Hormones and pheromones:

Hormones act between cells within an organism. Pheromones act between different organisms.

Hormones and secondary messengers:

Hormones act between different cells. While secondary messengers act within a cell.

Hormones and growth factors:

Hormones act far from where they are secreted, while growth factors act on cells close to their site of secretion.

Hormones and neurotransmitters:

Hormones can act far from where they are secreted and on many target cells. Neurotransmitters are secreted onto a neighboring cell.

32.) B.
33.) (See figures 46.11 and 46.12 in our text.) B.
34.) (See fig. 46.14, on pg. 1011 of our text. In some cases a range of times are possible.)

Bleeding and sloughing of uterine lining

←—Menses—→ ←—Proliferation—→ ←—Secretory phase—→

0   5   C   10   A D 15   B 20 E   25

3
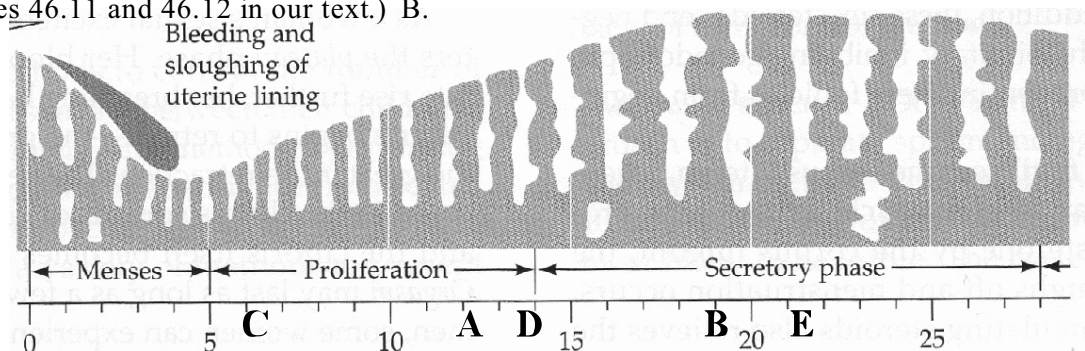
35.) (See pgs. 773-774 of our text.)
      2 Casparian strip.   1 A root hair.
      3 Vessel member in xylem tissue.   4 Cell wall of a mesophyll cell.
      5 Air inside of a leaf.

36.) B.
37.) C.

BIO 107   Final Exam   Summer 2010
Friday, July 9   Name: _____
         Points earned: _____ = _____ for course.
                                      145
Please keep your answers in the spaces provided as answers outside of there will not be read!
Good luck!

1.) Whether we hope to support a null hypothesis or not, what is gained by the use of the null hypothesis in the scientific method? (2 pts)

2.) Which is LEAST supportive of the Darwinian model of biological evolution? (1 pt)

    a.) Individual members of a species have various traits.
    b.) Instead of being tens of thousands of years old the earth is actually billions of years old.
    c.) Proteins can catalyze reactions over and over again.
    d.) The number of young produced by many species exceeds the number that a local area can support.
    e.) Individuals with some traits are more likely to survive to reproduce than individuals with different traits.

      Answer: _____

3.) Consider the carbonyl, carboxyl and hydroxyl functional groups. Which of the following statements CORRECTLY describes a feature of these functional groups? (2 pts)

    a.) Only the carboxyl and hydroxyl can participate in H-bonds with water.
    b.) All three of the groups often ionize when in the cytosol of cells.
    c.) All carbonyl groups are made up of carbons with two bonds to an oxygen with its other two bonds made with two other carbons.
    d.) All three groups are capable of participating in H-bonding interactions with water, but only the carboxyl group will normally ionize in the cytosol of cells.
    e.) The carbonyl group is unlikely to promote solubility in water because it cannot participate in H-bond formation.

      Answer: _____

4.) For each of the following give a specific name of an example of a monomer and a polymer. (6 pts)

Class of Organic Matter	Example of a monomer	Example of a polymer
Protein		
Carbohydrate		
Nucleic Acid		

5.) The following figure shows the relative free energy state of reactants and products of two reversible reaction cycles. Four reaction pathways (1-4) are indicated in the figure. (7 pts)

a.) Identify the reaction (both substrates and products) that has the largest net change in free energy.

b.) Which reaction pathway(s) has/have most likely been catalyzed by an enzyme?
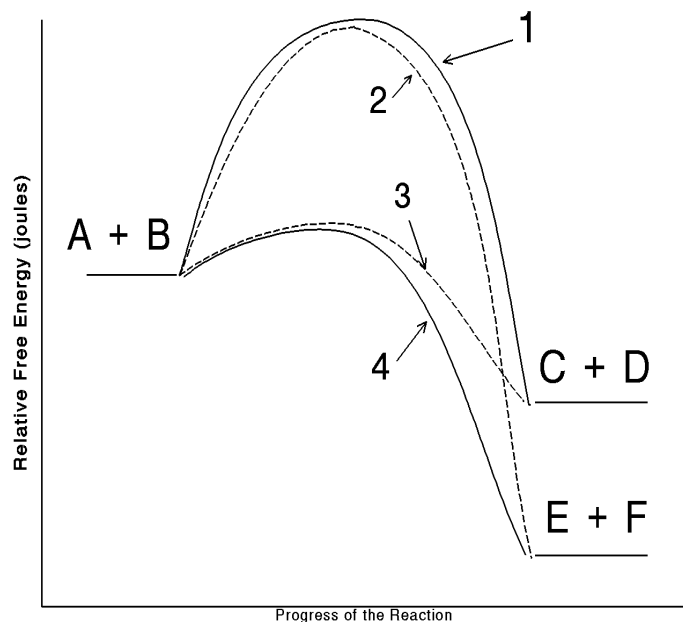
c.) Of the four reaction pathways shown:
       The one that occurs at the slowest rate is most likely _____ .

       The one that occurs at the fastest rate is most likely _____.
d.) On the figure draw a bracket to label the full extent of an activation energy barrier for an ENDERGONIC reaction.

6.) Select three (just 3!) of the following pairs of items (indicate the letter for each choice), and for each you select indicate a feature or ability of the first item in the pair that the second lacks or does not do. (3 pts)

        a.) Tight Junctions versus Gap Junctions.
        b.) Microtubules versus Intermediate Filaments.
        c.) Cristae versus Thylakoids.
        d.) Nucleolus versus Nuclear Lamina.
        e.) Plasma membrane versus Cytosol.

letter =

letter =

letter =

7.) A sodium ion/glucose symport system is often used by animal cells to take up glucose. Thus glucose molecules are typically moved by this system from a lower concentration outside to a higher concentration inside the cell. The sodium ions that are moved into the cell are moved to a lower concentration and into an area that is more negatively charged. Is this transport system overall a net active or a net passive system? Justify the reasoning behind your answer. (4 pts)

8.) Match each of the items with the appropriate letter to indicate a pathway (just one) that makes it as a PRODUCT. (5 pts)

    C Citric Acid Cycle   F Fermentation
    G Glycolysis   P Pyruvate import

    _____ $FADH_2$ _____ Acetyl-CoA   _____ Pyruvate

    _____ NAD^+ _____ CO_2

9.) Which is an INCORRECT statement about the normal processes carried out by the inner mitochondrial membrane? (2 pts)

     a.) Across it the process of chemiosmosis occurs.
     b.) There are redox reactions that are catalyzed by the ATP synthase located in it.
     c.) Oxygen gas acts to accept electrons from items in it.
     d.) Either $FADH_2$ or NADH can act as electron donors to items in it.
     e.) It acts as a barrier to maintain a proton electrochemical gradient.

        Answer: _____

10.) In carrying out photosynthesis most plants lose vast amounts of water from their bodies. What is the reason for this high water loss by plants that are doing photosynthesis (i.e. what is the major benefit plants obtain from this water loss)? (3 pts)

11.) The purpose of information transduction in cells can best be said to be to: (1 pt)

     a.) Allow the cell to sense various environmental stimuli.
     b.) Alter the expression of genes in the cell.
     c.) Provide a means by which the cell can phosphorylate many of its proteins.
     d.) Keep the concentration of Ca^{+2} in the cytosol low.
     e.) Connect the reception of a signal to the initiation of a response made by the cell.

        Answer: _____
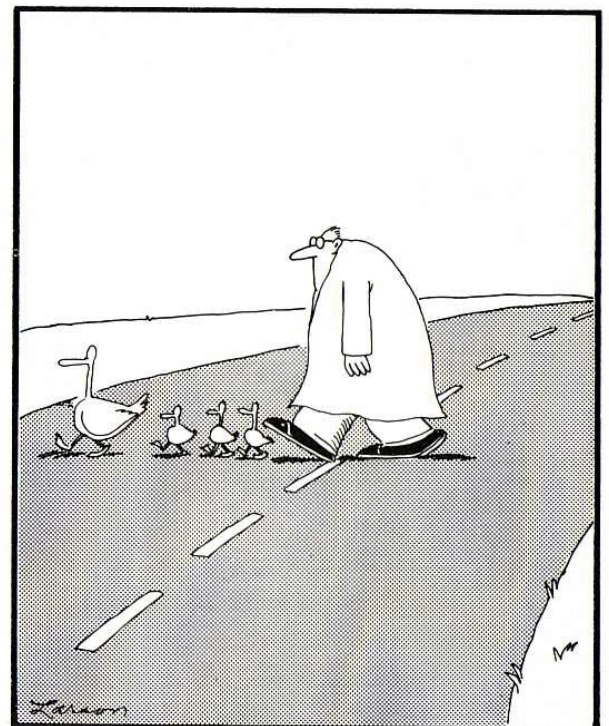
When imprinting studies go awry

12.) Select two (just 2!) of the following. For each item you select: Describe the major function of the item, and identify an example of one specific protist that has the trait. (4 pts)

contractile vacuole	
micronuclei	
coenocytic	

13.) Identify three typical animal tissues, and for each note a distinctive cell type that is found in it. (6 pts)

Tissue: Cell type:

14.) Which is **NOT** a feature that helps animals to avoid digesting themselves? (2 pts)

a.) They digest items in a cellular compartment other than their cytosol.
b.) They secrete mucus to protect their cells from digestive enzymes.
c.) They mainly take up monomers, which need little further digestion, into their cells.
d.) Their digestive enzymes are allosterically activated when in their cytosol.
e.) Cells lining their digestive tract have tight junctions to prevent leakage into the body spaces.

Answer: _____
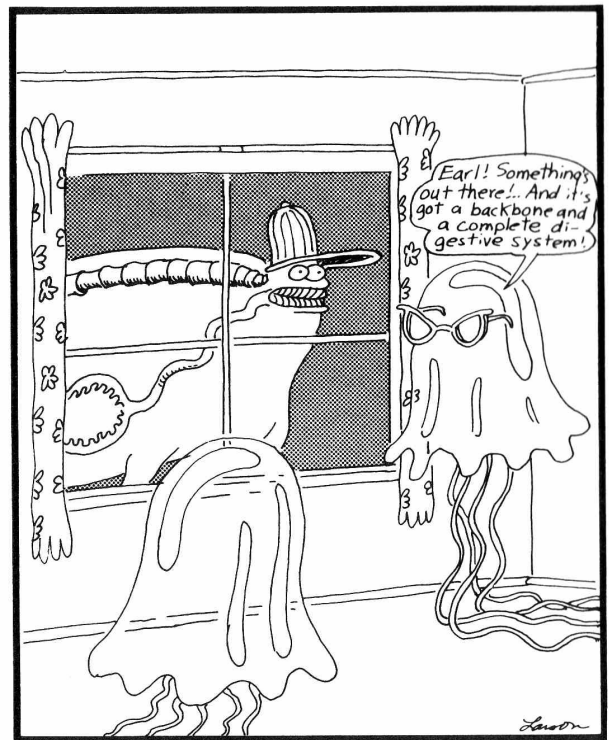
Life in the primordial soup

15.) If the human heart is beating properly, then starting from a fully relaxed heart the **FOURTH** event to occur would be: (2 pts)

   a.) An electrical impulse travels through the sinoatrial node.
   b.) After a short delay an electrical impulse travels down the atrioventricular node.
   c.) The atrioventricular valves close, and the semilunar valves open as blood flows through them.
   d.) The two atria contract, sending blood through the atrioventricular valves.
   e.) Contraction of the ventricles begins at the posterior end of the heart.

   Answer: _____

16.) The following figure shows the percent of oxygen saturation of several possible oxygen binding proteins under different situations. Which choice INCORRECTLY identifies a likely molecule and situation for its corresponding lettered curve in this figure? (2 pts)

   a.) Myoglobin in the muscle cells.
   b.) Fetal hemoglobin.
   c.) Adult hemoglobin in capillaries in the lungs.
   d.) Adult hemoglobin in capillaries in the liver.
   e.) An isolated single subunit of adult hemoglobin.

   Answer: _____
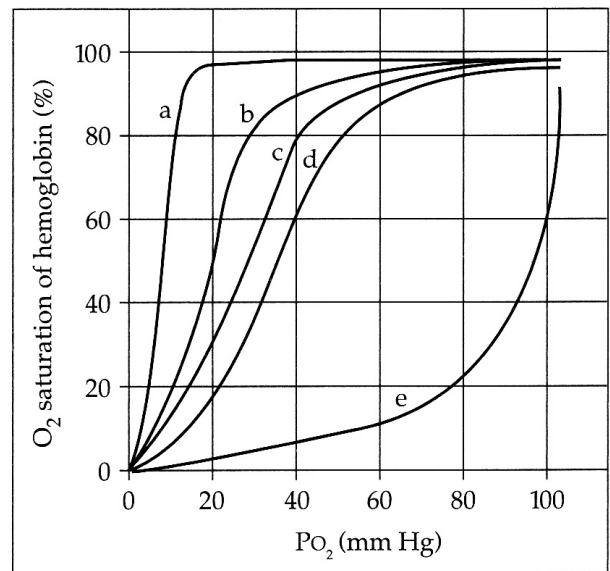
17.) In order to get the K^+ it needs into its cells a plant would most likely use which one of the following mechanisms? (1 pt)

   a.) Take up extracellular solution via phagocytosis.
   b.) Use a H^+/K^+ symport system powered by the electrochemical gradient of protons across the plasma membrane.
   c.) Take up K^+ via a K^+-pumping ATPase in their plasma membranes.
   d.) Use a Na^+/K^+ symport system powered by the electrochemical gradient of Na^+ across the plasma membrane.
   e.) Actively accumulate K^+ through use of an ion channel in the plasma membrane.

   Answer: _____

18.) Which best completes the following sentence about nitrogenous waste products? _____ is the type of nitrogenous waste that requires the most energy to produce, while _____ is another type of nitrogenous waste that requires the greatest amount of water to be excreted. (1 pt)

        a.) Uric Acid, ammonia
        b.) Urea, uric acid
        c.) Ammonia, uric acid
        d.) Uric Acid, urea
        e.) Urea, ammonia

        Answer: _____

19.) If you arranged the following items found in skeletal muscle tissue from biggest (i.e. most inclusive) to smallest (i.e. least inclusive) in size, which component would be **FOURTH** in order? (2 pts)

        a.) Thick filament
        b.) Muscle fiber
        c.) Myosin headgroup
        d.) Myofibril
        e.) Sarcomere

        Answer: _____

20.) Which of the following is not a modification seen in some plant species for promoting an ABIOTIC means of dispersal of its next independent generation? (2 pts)

        a.) Increase the size and buoyancy of the fruit coat so that it floats for a long time on water.
        b.) Make the seed coat hard and resistant to the conditions found in an animal's digestive tract.
        c.) Grow the fruit coat to be broad, flat, and light in mass so that it is easily moved by the wind.
        d.) As it dries have the fruit coat become tense so that it explosively snaps and expels the seeds.
        e.) Reduce the mass of the seed in the fruit and cover the fruit with long feathery hairs and fibers.

        Answer: _____

21.) Together thyroid hormones (T3 and T4) and the steroid hormones as a group are different in how they function compared to those hormones that are derived from entire proteins. Which one of the following statements BEST describes an important difference between these two groups of hormones? (2 pts)

      a.) Protein-based hormones travel long distances through the blood, while these thyroid and steroid hormones act close to where they are released.
      b.) T3, T4, and steroid hormones have short term effects, while protein-based hormones have longer term effects.
      c.) The protein-based hormones act in positive feedback systems, while the thyroid and steroid hormones act in negative feedback systems.
      d.) T3, T4, and steroid hormones may have receptors inside of, while protein-based hormones typically have receptors on the surface of, their target cells.
      e.) T3, T4 and steroid hormones bind to other hormones before they have their effects, while protein-based hormones do not have to bind to other hormones to act.

        Answer: _____

22.) Write the letter for the proper hormone with the phrase that it best matches. (5 pts)

      a.) Estrogen   b.) Progesterone
      c.) Luteinizing hormone   d.) Follicle-stimulating hormone
      e.) Human chorionic gonadotropin

      _____ This hormone stimulates ovulation and development of the *corpus luteum*.
      _____ This hormone is produced by the developing follicle and promotes the initial thickening of the endometrium.
      _____ This hormone is produced by the growing embryo, and has effects in the mother.
      _____ This hormone stimulates sperm production.
      _____ This hormone is made by the *corpus luteum*, and later in pregnancy is made by the placenta.

23.) Which one of the following best describes how a plant generates root pressure? (1 pt)

      a.) Ions are taken up at a net cost of energy to the plant and dumped into the xylem.
      b.) The cells in the root swell pushing water in the root upward.
      c.) Water is lost from the leaves and this pulls water up to the leaves.
      d.) Water is actively pumped past the Casparian strip to the xylem in the center of the root.
      e.) Osmotically active items are accumulated in the phloem, generating osmotic pressure.

        Answer: _____

24.) There are three gases that are typically exchanged through the stomatal pores of leaves for reasons that are ADAPTIVE to the plant. These three gases are: (1 pt)

        a.) O_2, CH_4, H_2O.
        b.) CO_2, N_2, O_2.
        c.) H_2O, CO_2, N_2.
        d.) O_2, CO_2, CH_4.
        e.) H_2O, CO_2, O_2.

           Answer: _____

25.) The mammals and birds that are living today share all the following features EXCEPT for which one of the following? (2 pts)

        a.) An amniotic egg, or structures derived from the amniotic egg.
        b.) They are mainly endotherms, and use items extruded from their skin to help with thermal regulation.
        c.) Production of some form of milk used to feed their young.
        d.) A muscular, post-anal tail of some sort at some point in development.
        e.) A closed circulatory system with a four-chambered heart.

           Answer: _____

26.) Pick one (1!) of the following traits and for it identify a structure into which it has been modified and note in which animal group it is thought that this modification first evolved. (3 pts)

Traits	Modified into:	Animal Group:
Pharyngeal Slits		
Extra-embryonic membranes		
Fleshy Fins		

27.) A bony ray-finned fish (Actinopterygii), such as a trout, is in which of the following groups? (check all that apply) (5 pts)

___ Mammals   ___ Deuterostomes   ___ Craniates   ___ Tetrapods

___ Gnathostomes   ___ Vertebrates   ___ Amniotes   ___ Chordates

___ Lobe-finned fish   ___ Osteichthyans

9

28.) Below are the skeletons of forelimbs of various vertebrates. For parts (a-c) indicate with the appropriate letter which skeleton is likely to be found in an animal that would use the indicated type of locomotion. For each describe reasons in support of your choice. (10 pts)

a.) Which skeleton is most likely involved in digging? _____ Describe your reasoning:

b.) Which skeleton is most likely involved in flight? _____ Describe your reasoning:

c.) Which skeleton is most likely involved in running? _____ Describe your reasoning:

d.) Of all the joints in these skeletons which is a joint with the highest mechanical advantage? Indicate your choice of joint by drawing relative to it a plausible extensor muscle, clearly label the origin of the muscle (O), and the insertion point of the muscle (I) relative to the joint.

A.)

B.)

C.)
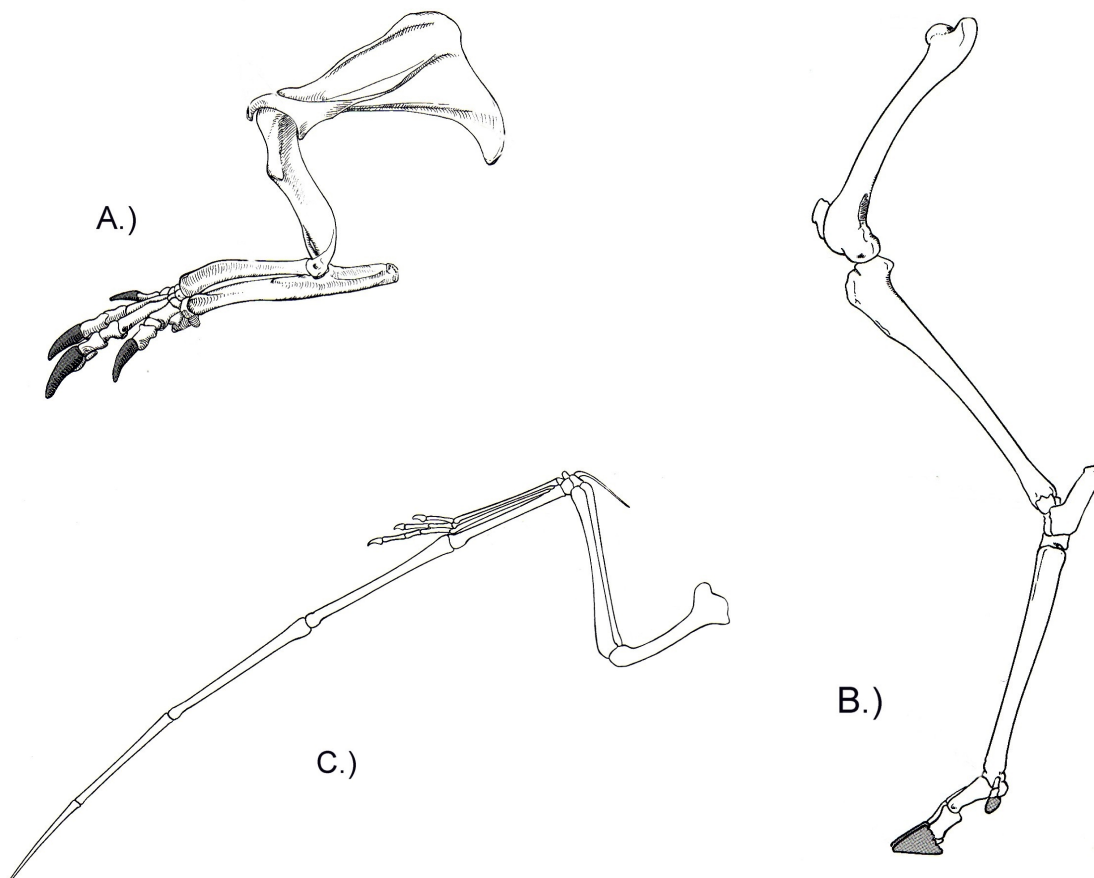
29.) Insulin is a human hormone, and it has numerous receptors in various locations of our bodies. Identify a typical location of insulin in our bodies, and one typical location of an insulin receptor. Contrast these two molecules in terms of a difference in one of their properties pointing out how this difference is consistent with the different locations you noted. (5 pts)

30.) If you wish to separate DNA from starch, what is one technique that would be helpful? Explain the reasoning behind your answer by noting the physical properties that the technique uses to separate these two sets of molecules. (3 pts)

31.) In a normal action potential in a human neuron which one of the following events is most likely to occur **second**? (2 pts)

     a.) The neuron's membrane potential will hyperpolarize.
     b.) Sodium channels in the neuron's membrane are activated.
     c.) Potassium ions move out of the cell.
     d.) The neuron's membrane potential will depolarize.
     e.) Potassium channels in the neuron's membrane are activated.

Answer: _____

32.) For an action potential to move from one node to the next down an axon what travels between the nodes? (2 pt)

    a.) The Na^+ moves from nodes nearer the cell body down the axon to the next node.
    b.) The electrical field generated at one node travels to the next node and influences the membrane potential there.
    c.) Potassium ions move out of the cell at one node, and flow outside of the cell to enter at the next node further down the cell.
    d.) The electric field at one node pushes Na^+ and K^+ down the length of the cell, forcing an accumulation of positive charges at the next node.
    e.) Neurotransmitters released at one node are detected by ligand-gated ion channels at the next node, and this induces an action potential to be generated at this next node.

    Answer: _____

33.) Whether they are in animal neurons, or muscle cells, or in algae, what are two features that we would expect to see in an action potential that would help us to recognize it as an action potential? (2 pts)

34.) A cell has a membrane potential of -70 mV when at rest. The movements of different ions across its membrane can alter the membrane potential in several ways. Which one of the following describes a movement of ions which will result in the DEPOLARIZATION of this cell's membrane potential?
(1 pt)

    a.) Potassium ions (K^+) moving into the cell.
    b.) Chloride ions (Cl^-) moving into the cell.
    c.) Sodium ions (Na^+) moving out of the cell.
    d.) Hydrogen ions (H^+) move in while potassium ions move out of the cell.
    e.) Calcium ions (Ca^{+2}) move out of the cell.

    Answer: _____
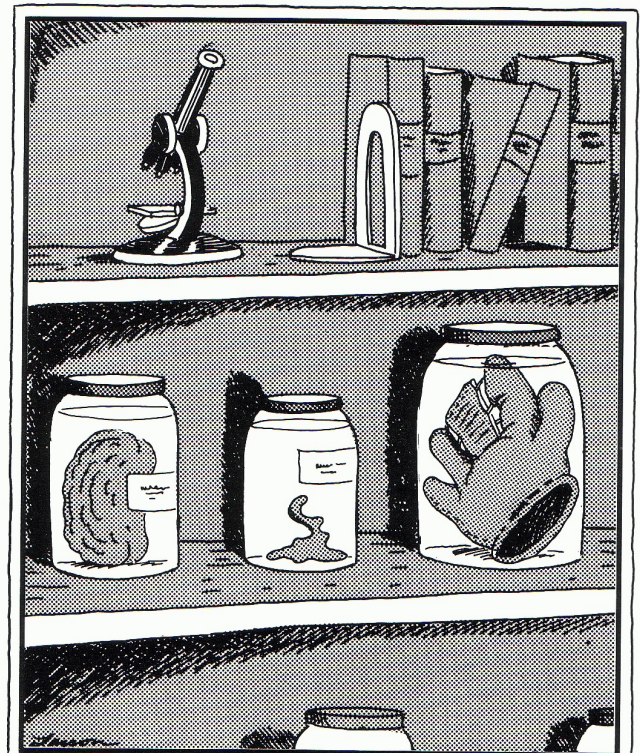
Broca's brain, appendix, and baseball glove

35.) After a synapse has been used it must be reset to its original condition so that it can be used to transmit another signal. Which one of the following INCORRECTLY describes something that will need to be reset in this synapse?   (2 pts)

a.) The concentration of neurotransmitter in the synaptic cleft must be lowered.
b.) More vesicles containing neurotransmitter in the pre-synaptic cell must be moved
     close to the synapse.
c.) The voltage-sensitive Ca^{+2} channels in the pre-synaptic cell must be closed.
d.) The concentration of Ca^{+2} in the pre-synaptic cell's cytosol near the synapse must be
     lowered.
e.) The ligand-gated ion channels in the pre-synaptic membrane must be closed.

Answer: _____

36.) Which BEST completes the following? An inhibitory postsynaptic potential (IPSP) is typically produced when: (1 pt)

a.) sodium ions diffuse into the post-synaptic cell.
b.) the binding of neurotransmitters causes the membrane of the post-synaptic cell to
     depolarize.
c.) the neurotransmitter in the synaptic cleft is present for a long period of time.
d.) an action potential crosses an electrical synapse.
e.) chloride ions diffuse into the post-synaptic cell.

Answer: _____

37.) For the following items indicate by marking the appropriate box whether it is typically found in the pre-synaptic side of a chemical synapse, or the post-synaptic side of a chemical synapse. Also rank the three items given from first (1) to last (3) use when this synapse is used. (6 pts)

Is it in?		Item:	Likely order of use. (1-3)
Pre-Synaptic side of the synapse.	Post-Synaptic side of the synapse.		
		Voltage-sensitive Ca^{+2} channels	
		Ligand-gated channels	
		Synaptic vesicles	

38.) In the human knee-jerk response which of the following events typically occurs **last**? (2 pts)

       a.) An action potential leaves the ventral side of the spinal cord.
       b.) A sensory neuron receives an excitatory stimulus.
       c.) An action potential arrives at the dorsal side of the spinal cord.
       d.) An action potential arrives at a neuro-muscular junction.
       e.) An interneuron receives information and fires off an action potential.

       Answer: _____

39.) Consider the sympathetic and parasympathetic divisions of the human nervous system. (5 pts)

      Are these part of the **Central Nervous System** or the
           Peripheral Nervous system ? (Circle your choice.)

      Describe a major structural difference between the sympathetic and the parasympathetic
          divisions.

      Select one of the following organs. (Circle your choice.)

         Gallbladder   Pancreas   Salivary glands

      For the organ you select, describe how the effect on this organ by neurons of the
          sympathetic division differs from the effect resulting from the action of neurons of
          the parasympathetic division.

40.) Select two (just 2!) of the following items found in vertebrates. For each identify a specific function it carries out, or a distinctive structural or locational feature that it has. (4 pts)

Hindbrain:
White matter:
A motor neuron:
Cerebrum:
Medulla oblongata:

41.) Match each of the following structures with the letter of the proper mammalian sense. (5 pts)

     S   sight   H   Hearing   T   Taste

_____ Tight gap junctions limit the diffusion of extracellular ATP.
_____ Hair cells are used.
_____ Bipolar cells are present in this sensory organ.
_____ Intracellular disks modified from the endoplasmic reticulum are present.
_____ A round window is present in this sensory organ.

42.) Fill in the following blanks with the best word or phrases. (3 pts)
Rhodopsin absorbs a photon of light, this causes the retinal to isomerize.   In mammals this occurs in

a cell called a _____ cell. The next event to occur is likely to be the release

of an active _____ that will bind with phosphodiesterase and active it so that

_____ can be cleaved.

43.) A set of sensory cells in one of our taste buds is about to receive a signal. Which of the events listed below is most likely to occur THIRD as the signal is received and transduction occurs? (2 pts)

      a.) A potassium ion channel is now unable to allow ions to pass through it.
      b.) Vesicles fuse to the cell's plasma membrane.
      c.) ATP is released into the extracellular space.
      d.) A receptor in a cell undergoes a change in its shape.
      e.) The concentration of cAMP in at least one part of the cytosol rises.

          Answer: _____

44.) You calculate that in a cell in your body the energy in molecular motion is in the range of 4×10^{-21} Joules. Which choice BEST describes how the energy in this thermal background noise compares to aspects of signals that can be detected by your body? (2 pts)

      a.) Any signal must have just this much energy so that it can get molecules to move.
      b.) Signals your body can detect can have this level of energy if they are able to pass through your body.
      c.) Only those signals that halt this molecular motion will be detectable by your body.
      d.) Any signal that can be detected must be able to break covalent bonds, something that this thermal background noise does not typically do.
      e.) Detectable signals must have more than this background energy, and must be able to induce changes in the structure of certain molecules.

          Answer: _____

45.) In what way is the information in the signals sent by sensory neurons altered to carry the information that a detected environmental signal's amplitude is getting greater? (2 pts)

46.) Two types of environmental signals that humans are not noted to have the ability to detect are: (2 pts)

_____   _____

47.) A freshwater fish would be expected to: (1 pt)

    a.) pump salt out through salt glands in its gills.
    b.) produce copious quantities of dilute urine.
    c.) release urea across its gills.
    d.) have scales to reduce water loss to the environment.
    e.) use uric acid as a nitrogenous waste.

    Answer: _____

48.) The major reason that uric acid is used by birds is that uric acid: (1 pt)

    a.) has low toxicity and is easily washed away.
    b.) allows the developing chick in the egg to conserve water.
    c.) is used to strengthen the egg shell.
    d.) is inexpensive to produce.
    e.) can be used as an osmotically active substance for osmoregulation.

    Answer: _____

49.) One feature that IS found in the circulatory system of many amphibians, but that is NOT found in the circulatory system of most fish, is the: (1 pt)

    a.) division of the ventricle into right and left chambers.
    b.) presence of one-way valves in their veins.
    c.) compete separation of oxygenated and deoxygenated blood.
    d.) division of the atrium into distinct right and left atrial chambers.
    e.) capillary beds associated with the skin of the animal.

    Answer: _____

50.) Which choice INCORRECTLY completes the following? Most of the blood leaving the heart of a _____ will pass through _____ capillary bed(s) before returning to the heart. (2 pts)

    a.) trout; two
    b.) frog; two or three
    c.) crocodile; one or two
    d.) bird; one
    e.) dog; one

    Answer: _____

51.) When a cold-adapted endotherm, such as an arctic wolf, walks on ice: (1 pt)

    a.) the warm blood from the wolf's body is carried out in arteries near the skin surface to keep the feet warm.

    b.) a counter current exchange mechanism warms the blood coming from the feet to return heat to the body's core.

    c.) the temperature of its feet is maintained at the same temperature as the rest of the body by a counter current exchange mechanism.

    d.) the blood in the veins is cooled by the blood in the arteries by a counter current exchange mechanism.

    e.) a counter current heat-exchange mechanism warms the blood going to the feet.

       Answer: _____

52.) Select two (just 2!) of the following terms, and for each define the term and describe one specific example of it. (4 pts)

Habituation
Sign stimulus
Operant conditioning
Altruistic behavior

53.) Which pair of individuals do NOT have a coefficient of relatedness of 0.5? (1 pt)

     a.) A father and his daughter.
     b.) A mother and her son.
     c.) An uncle and his nephew.
     d.) Two brothers.
     e.) A sister and her brother.

        Answer: _____

54.) Sow bugs (a small arthropod) become more active in dry areas, moving more and faster, and less active in humid areas. This behavioral change is an example of a: (1 pt)

     a.) taxis.
     b.) tropism.
     c.) kinesis.
     d.) cognitive ability.
     e.) net reflex.

        Answer: _____

55.) In behaviors the role played by neurons in animals is similar to the role played by _____ in a single-celled organism. (2 pts)

     a.) receptors on the surface of the cell
     b.) a protein phosphorylation cascade in the cytosol of the cell
     c.) genes
     d.) the Na^+/K^+ pumping ATPase
     e.) the contractile vacuole

        Answer: _____

56.) What distinguishes a growth response from a behavioral response? (3 pts)

57.) Give one example of a behavior that is seen in a plant. Describe the essential features of this example that make it consistent with being a behavior. (3 pts)

Optional Bonus Question. Any points earned in an answer to this question will be substituted for up to five missed points out of the 145 points that this exam is worth. (Please limit your answer to this side of this page.)

Describe three ways that a synapse between two interneurons that results in an IPSP in the post-synaptic neuron is either similar or different from a synapse between a motor neuron and a skeletal muscle cell. Also note: Which is more likely to play a role in information integration, and why? Which of these two synapses is more likely to be able to be altered? For it describe a possible change noting its main effect on that synapses' functioning.

BIO 107   2010
Final Exam   Proposed Answers:

Below are proposed answers for the questions in this exam.

Be aware that for many of these questions other answers exist that may also be acceptable either for full or partial credit.

Any comments about, or suggestions of alternate answers for, these questions should be sent to me via e-mail by noon on Saturday at the latest. I will attempt to take them into account as I do the grading of this exam.

1.) The null hypothesis states specific expectations, and it is very helpful to have a well defined set of expectations so that collected results can be evaluated.

2.) C.
3.) D.
4.)

Class of Organic Matter	Example of a monomer	Example of a polymer
Protein	glycine	actin
Carbohydrate	glucose	starch
Nucleic Acid	adenosine deoxyribose triphosphate	DNA

5.)
    a.) A + B = E + F
    b.) reaction pathways 3 and 4.
    c.)   Slowest: 1
            Fastest: 4
    d.) Endergonic reactions are ones going from the right back to the left of the figure.
            One bracket would be from the C + D energy position back up to the
            peak energy level in pathway #3.
6.)   (Only three answers were needed here.)
        a.) Tight junctions prevent items in the extracellular solution of animal tissue from passing between them. Gap junctions do not limit the movement of extracellular items.
        b.) Microtubules can act as a structure along which dynein motor proteins can generate force. Intermediate filaments do not have dynein work on them.
        c.) Cristae are membranes across which oxidative phosphorylation occurs, while these respiratory reactions are not done across thylakoid membranes.
        d.) The nucleolus is where ribosomal RNAs are made, processed, and initial assembly is done. The nuclear lamina is not a region of gene expression, but rather is a structural item.
        e.) The plasma membrane has a membrane potential across it which can be a significant energy gradient. The cytosol has no such energy gradient.

1

7.) This is a passive transport system. The sum of the energy change from the sodium ion movement with the energy change of the movement of glucose gives a net negative free energy change. This makes the overall result a spontaneous change, and so passive.

8.)

| C | $FADH_2$ | P | Acetyl-CoA | G | Pyruvate |
| F | NAD^+ | C | CO_2 | | |
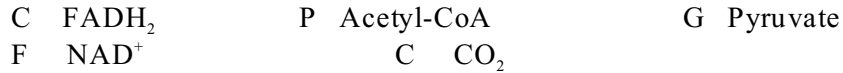
9.) B.

10.) By letting the water have the means to leave, the plants are also leaving a path open for carbon dioxide to enter. The carbon dioxide is needed by the plant for carbon fixation. Thus the benefit the plant gains is access to a major item needed for photosynthesis.

11.) E.

12.)   (Only two answers were needed here.)

   Contractile vacuole: This removes excess water and recovers desired osmotica back to the cytosol. It is seen in paramecia.

   Micronuclei: This stores a copy of the genetic material for use in sexual recombination. It is seen in the ciliates, such as stentor.

   Coenocytic: A common cytosol has many nuclei. This is seen in the acellular slime molds such as plasmodium.

13.)

Muscle tissue:	Skeletal muscle fiber.
Nervous tissue:	Glia cells.
Connective tissue:	Red blood cells.

14.) D.
15.) E.
16.) E.
17.) B.
18.) A.
19.) A.
20.) B.
21.) D.

22.) The order down the phrases would be: C, A, E, D, B.

23.) A.
24.) E.
25.) C.

26.)   (Only one row needed to be answered in this case.)

Traits	Modified into:	Animal Group:
Pharyngeal Slits	Jaws	Gnathostomes
Extra-embryonic membranes	Placenta	Placental Mammals
Fleshy Fins	Limbs	Tetrapods

27.) Those that should be checked include: Deuterostomes, Craniates, Gnathostomes, Vertebrates, Chordates, Osteichthyans.

28.)
      a.) A. This skeleton has thick bones that could resist the high compression forces typically encountered with digging.
      b.) C. This skeleton has light and thin bones far from the base of the limb. Lighter bones can be moved faster than larger heavy bones. If a fast speed of flapping is needed for flight, then lighter bones are better as they can be moved faster.
      c.) B. This skeleton has a thick flat hoof that would be able to withstand the stress of running.
      d.) In skeleton A, the elbow joint has a high mechanical advantage. The extensor muscle would likely have an origin at the at the upper shoulder and insert on the extended end of the elbow bone.

29.) Insulin is typically secreted into the blood serum, and so is found in the blood of our body. The receptor for insulin would be found in the plasma membrane of certain cells of the body, such as some liver cells, that have access to the blood serum. Having to be in the watery blood serum, insulin is hydrophilic and highly water soluble. In contrast, the insulin receptor sits in the cell membrane and so has a hydrophobic core and is rather insoluble.

30.) DNA has a net negative charge, while starch typically has no charge. Thus electrophoresis could be used to pull the DNA molecules away from the starch molecules as the DNA would move when exposed to an electrical field while the starch would not.

31.) B.
32.) B.

33.)
      Action potentials in a species have:
      A characteristic length,
      and a characteristic amplitude.

34.) A.
35.) E.
36.) E.

37.)

| Is it in? | | | Likely order of use. |
Pre-Synaptic side of the synapse.	Post-Synaptic side of the synapse.	Item:	(1-3)
X		Voltage-sensitive Ca^{+2} channels	1
	X	Ligand-gated channels	3
X		Synaptic vesicles	2

38.) D.

39.)   Peripheral Nervous System

The sympathetic system has ganglia associated with it down the length of much of the spinal cord. The parasympathetic system has no such ganglia.

For the pancreas, the sympathetic system inhibits the pancreas from secreting digestive enzymes. In contrast the parasympathetic system would stimulate the pancreas to secrete more digestive enzymes.

40.)   (Only two answers were needed here.)
Hindbrain: The cerebellum of it coordinates motor actions of our bodies.
White matter: Regions of our central nervous system that have a high amount of myelinated axons passing through it.
A motor neuron: Has a synapse onto an effector cell, such as a cell in a muscle or a gland.
Cerebellum: A part of the forebrain that processes information and plans actions.
Medulla oblongata: A part of the hindbrain where sensory inputs are sorted and passed to other parts of the brain.

41.) The letters down the list of structures should be: T, H, S, S, H.

42.) ... rod... G protein... cGMP....

43.) B.
44.) E.

45.) The frequency of action potentials sent down the sensory neuron would likely rise.

46.)   magnetic fields ; X-rays

47.) B.
48.) B.

49.) D.
50.) B.
51.) B.

52.)   (Only two answers were needed here.)

     Habituation: After initially responding to a stimulus with a behavior, repeated exposure to the stimulus ultimately fails to elicit a response any more. A repeated alarm call by others of one's species can eventually result in no response to the alarm.

     Sign stimulus: A signal that initiates a strong and stereotypical response. An example would be the red belly of stickleback fish which stimulate male fish to attack the another male with a red colored belly.

     Operant conditioning: A rare event, but which has strong costs or benefits, can be recognized and features about it learnt so that it affects future behavior. This is called trial-and-error learning. An example would be a wolf attacking a porcupine and getting quills in its face, the wolf only has to do this once to learn not to attack porcupines.

     Altruistic behavior: An individual pays a cost or takes a risk, but to the benefit of others who are their relatives. A female ground squirrel attracts predators to herself by making alarm calls that warn its relatives far from the burrow of the presence of the predators.

53.) C.
54.) C.
55.) B.

56.) A growth response occurs just once, and results in the formation of new structures. While a behavioral response can be repeated and makes use of existing structures.

57.) The opening of stomates in the leaf of a plant is done each day. This is repeated daily, and makes no new structures. This behavior is in response to stimuli, it is modified by the daily weather and water status of the leaf, and is adaptive to the plant.

ACKNOWLEDGEMENT

INTRODUCTION TO THE BIOLOGICAL SCIENCES 1107-1108
LABORATORY COURSE

THE MAJOR CONCEPTS OF BIOLOGY

    Biology is described as the study of living things. An introductory course in biology includes such a huge collection of facts and new vocabulary that it is often difficult for students to grasp the meanings and relationships of the major concepts and principles of this field. Students frequently report that they have difficulty in relating the laboratory to the lecture course, and even in relating one day's laboratory to the next. Your major goals in this course should be to make connections among the concepts you are learning, to relate new information to what you already know, and to achieve a good basic understanding of biology.

    Figure 1 is a schematic representation of the "big picture" in biology; i.e., how the major concepts in the biological sciences are related. Such a diagram is known as a concept map, and is one of the tools that you can use to organize, relate, and learn the information presented in this course.
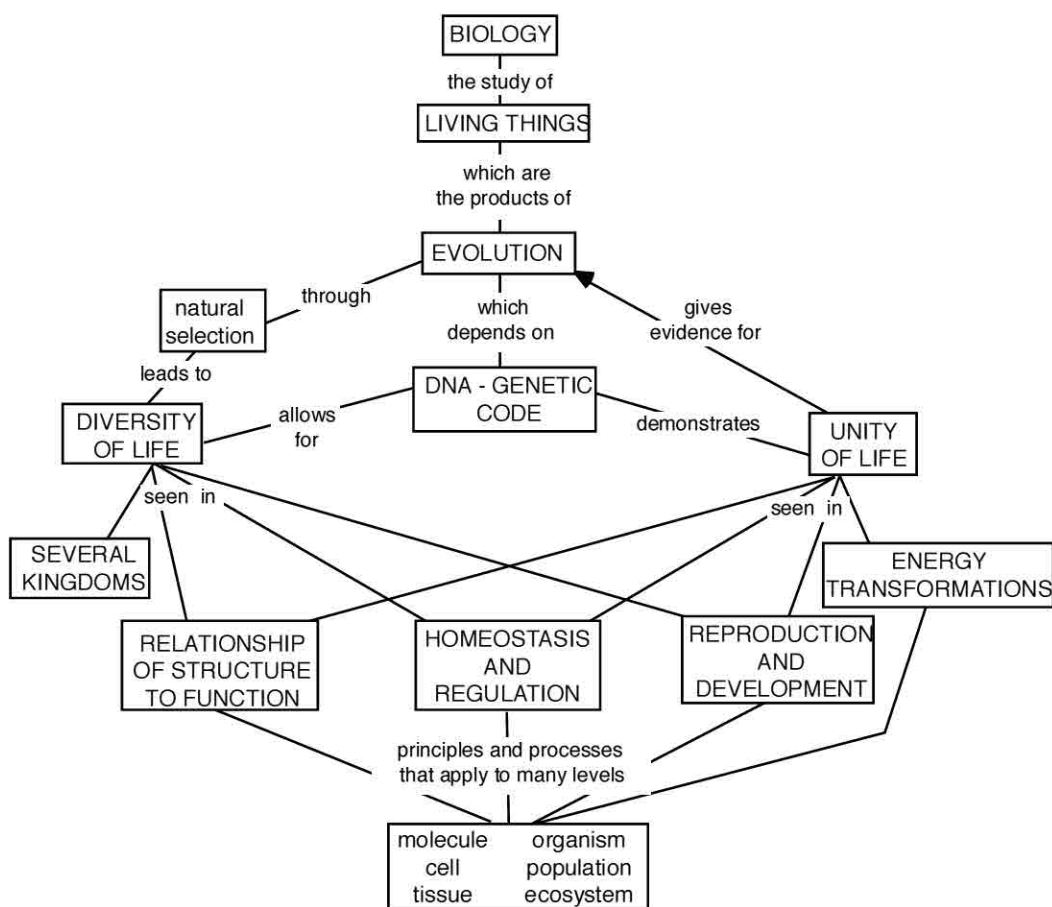
Figure 1. The "Big Picture" concept map of biology.

    In a concept map, the key concepts of a topic are arranged hierarchically, with the most inclusive or important ones at the top, and the more subordinate concepts underneath. Labeled lines are drawn between the concepts to illustrate and explain the relationships between and among concepts.

The concept map in Figure 1 presents one way of looking at and organizing the major ideas involved in the study of biology. **Evolution**, perhaps the most important guiding theory in modern biology, depends upon and acts through the universal genetic coding molecule, **DNA**. One way to look at the study of living things is to consider both the **diversity** and the **unity** of life. Biological diversity is quite obvious, as seen in the incredible variety of adaptations in structure, function, and life history seen both in evolutionary history and in the world today. The study of biology, however, will also emphasize the amazing unity of life, seen in DNA, energy transformations, reproduction and development patterns, mechanisms for regulation and homeostasis, and the relationship between structure and function. All of these major processes or attributes of living things can be considered on a variety of levels, ranging from molecular through the cellular, tissue and organ, organismal, population, and ecosystem levels.

As you go through the laboratory course, refer back to this concept map to see how each laboratory is related to 1) specific concepts of biology, 2) other laboratory topics, and 3) the concepts you are learning in the lecture course. This practice should help you to integrate the material you are learning in laboratory into your developing picture and understanding of the field of biology.

THE CONSTRUCTION OF SCIENTIFIC KNOWLEDGE

The "scientific method," the approach that scientists use in creating knowledge about natural objects and events, has often been described as a series of discrete steps, beginning with observations, and proceeding through question-asking, hypothesis-generating (using inductive reasoning), prediction-making (using deductive reasoning), hypothesis-testing (using either an observational or experimental design), and finally to analysis and interpretation of results. While it is doubtful that scientists always follow these exact steps in developing scientific knowledge, this generalized view of the "scientific method" points out some important components of knowledge construction.

Certainly an important first stage in making knowledge is to observe carefully and to ask good questions about some aspect of the natural world. What we have to remember, however, is that what we choose to observe and the kind of questions we ask are influenced by what we already know. We operate within an explanatory system of concepts, principles, and theories which has been developed over time through previous research and thought. New research serves to substantiate, refine, or expand this explanatory system. Occasionally the explanatory system must be dramatically revised when it can no longer adequately account for the results of new experiments or observations. Thus, scientific knowledge constantly grows and changes as our technology allows us to make new observations, and also as our developing knowledge allows us to ask and test new questions.

THINKING, LEARNING, AND WRITING SKILLS

The concept map in Figure 2 illustrates the three components of the learning objectives for this course. In your laboratory experience you will be dealing with the major concepts of biology and developing important lab skills. You will make and test hypotheses and learn how to transform, evaluate, interpret and present data. Communication skills are essential in science. This course will give you experience in reading and writing scientific papers. You can employ concept mapping to help you better prepare for lab, to guide your laboratory work, and to help you organize and learn the laboratory material. This tool is intended to help you rely less on memorization as an approach to learning this large quantity of material, and to focus more on developing your own personal picture and understanding of biology.

Martha R. Taylor

Revised June 2010
Mark A. Sarvary

Figure 2. Concept map for learning objectives in BioG 107-108.

TABLE OF CONTENTS
Bio G 1107
Summer 2010

CHAPTER 1 – BIOLOGY AS A SCIENCE

Jon C. Glase

LABORATORY SYNOPSIS

In this chapter you will explore the basic features of science and the process by which scientists construct new knowledge. The importance of accurate observations and the roles of both inductive and deductive reasoning to science are described, and experimental and observational tests of hypotheses are discussed. You will be applying the concepts developed in this chapter throughout the year, as you participate in the laboratory activities described in this book.

LABORATORY OBJECTIVES

1. Describe what an explanatory system is and identify how a scientist uses such a system in the interpretation of new observations.

2. Distinguish between the processes of inductive and deductive reasoning and show how each is used in the scientific method.

3. Describe observational and experimental tests of hypothesis, and identify the strengths and weaknesses of each.

4. Distinguish between a research hypothesis, a null hypothesis (H_o), and an alternative hypothesis (H_a).

5. Differentiate between observed results and expected results and explain how a comparison of observed and expected results tests the validity of a research hypothesis.

6. Describe the role of statistical testing in the scientific process.

INTRODUCTION

Biology is the scientific study of the phenomenon we call life. As a science, biology is in part a process and in part a collection of the products of the process. The scientific method is the process used by biologists to formulate generalizations about life that are reported in scientific journals and eventually are included in textbooks and presented in lectures. These generalizations must frequently be modified or discarded as biologists learn more about life. Part of your effort in studying biology will involve you in activities so you become more proficient in using the scientific method to formulate new generalizations about biology.

We must recognize at the outset that science can only be used to study phenomena that can be sensed. Ultimately our sense organs limit what we can learn about the world. In this regard, technology has been extremely important in developing instruments that extend what we can see, hear, or otherwise measure directly. We must also recognize a certain bias in how we, as humans, perceive the world. For example, if each of you were given a leaf and asked to describe it as completely as possible, the majority of your descriptions would probably be based on vision. Few of you would include information on how the leaf feels, or smells, or tastes because we are, by nature, a visually oriented species. How would a dog's description of the world (were we able to obtain it) be different from our own?

THE SCIENTIFIC METHOD

We shall describe the scientific process in three stages that reflect the chronology of how science is normally done. These stages illustrate the major types of activities in which scientists are involved.

1.   Observation and hypothesis formation

2.   Making predictions and testing hypotheses

3.   Analysis and interpretation

OBSERVATION AND HYPOTHESIS FORMATION

Ultimately, all scientific knowledge is based on observation of objects and events in the world, including things that may not seem relevant to one's immediate concern. The discovery of penicillin is an example of the importance of keen observation to science. The bacteriologist Alexander Fleming accidentally left a culture dish of staphylococcus bacteria exposed in the laboratory for several days. Before discarding the culture, Fleming noticed that a colony of green mold (a fungus) had started to grow on its surface. He also observed that a clear zone, where the bacterial cells were no longer growing, surrounded the fungus colony. Fleming hypothesized that the fungus, later identified as *Penicillium notatum*, must be producing a substance (now known to be the antibiotic penicillin) that killed the bacteria.

In your laboratory work in biology you will be refining your observational abilities. Since it is critical that your observations be both accurate and objective, writing a description of what you see and including drawings will help in this regard. Also, verifying your observations—assuring that you can make the same observations again under similar conditions—shows that the phenomenon is not a random event but represents regularity in the world.

After a scientist has verified a new observation, he or she tries to explain it in terms of what is already known about that area of science. The attempt to understand new phenomena by referring to verified explanations for related phenomena is a method of thinking used by all of us, both scientists and nonscientists. The information we refer to when interpreting a new observation is called an **explanatory system**. A scientist's explanatory system consists of verified results from previous research in a particular area. The tentative explanation for the new observation is called a **research** or **working hypothesis**. Formulating a research hypothesis involves a type of reasoning called **inductive logic**, in which we make a general statement that summarizes specific observations and attempts to make them consistent with an explanatory system.

To better understand the scientific process; let us examine how it was used in an important study of evolution conducted by the British biologist H. B. D. Kettlewell during the mid-1950s. Kettlewell and his co-workers were interested in understanding changes that had occurred in populations of the peppered moth (*Biston betularia*) in England. This moth exists in one of two different forms, or **morphs**: a light-colored morph and a dark-colored morph. By studying insect collections, Kettlewell noticed that prior to 1850 the majority of peppered moths in the Birmingham area of England were light morphs. However, based on many collecting trips, he and others observed that by the 1950s the dark morph was much more numerous than the light morph in the same area. This change in the relative numbers of the two morphs was the new observation that Kettlewell wished to explain.

The explanatory system that Kettlewell used to interpret his observations included the following established facts:

1. Moths, including the peppered moth, are active mainly at night, spending the day resting on trees and rocks.

2. If an organism like a moth has **cryptic coloration** (looks like its background), it will be more difficult for visually oriented predators to locate.

3. The tree surfaces in areas surrounding industrial regions (such as Birmingham) have become darkened, due to the accumulation of soot and the disappearance of light-colored lichens.

4. Coloration in moths is genetically determined.

Using this explanatory system and reasoning inductively, Kettlewell developed the following research hypothesis to explain the shift in the observed proportions of the light and dark morphs:

The increase in the dark morph is due to decreased predation because it is more difficult for predators to find them on the darkened bark surfaces (greater crypticity); the decrease in the light morph is due to increased predation because of reduced crypticity on the darkened bark surfaces.

MAKING PREDICTIONS AND TESTING HYPOTHESES

The next stage in the scientific method is to determine if the research hypothesis explains the new observation and makes it compatible with what is already thought to be true. We proceed by determining what predictions the research hypothesis allows us to make and then we conduct a study to discover if these predictions are true. The results from the study, then, indirectly test the validity of the original hypothesis.

The thinking process that is used to make predictions from a research hypothesis is called **deductive logic** which always takes the form

> *If . . . (statement of research hypothesis)*
> *then . . . (statement of prediction)*

Let us put Kettlewell's research hypothesis in this form and examine two of its predictions:

> *If the increase in the dark morph is due to decreased predation because of their increased crypticity on the soot-darkened bark surfaces, and the decrease in the light morph is due to increased predation because of their reduced crypticity on the soot-darkened bark surfaces*

> *then (1) we should be able to collect significantly more light moths than dark moths from soot-free woodlands in unpolluted areas; and (2) we should be able to collect significantly more dark moths than light moths from soot-darkened woodlands near industrial areas.*

These predictions indicate what research needs to be done to test the hypothesis.

The actual research activity whereby the data are collected is a step in the scientific process called the **test of hypothesis**. Tests of hypothesis are of two types: **observational** and **experimental**. An observational test (such as the one described above) involves making additional new observations that should be possible to make if the research hypothesis is valid. An experimental test involves manipulating the environment in some way, rather than just making observations. Examine the data collected by Kettlewell in two different woodland areas to test by observation his hypothesis (Table 1.1):

Table 1.1. Numbers and percent of light morphs and dark morphs of peppered moth (*Biston betularia*) collected in soot-free and soot-darkened woodland areas (Kettlewell, 1959).

Number (%) of Moths Collected

Morph Type	Soot-Free Woodland		Soot-Darkened Woodland	
Light	324	(99.1)	144	(9.3)
Dark	3	(0.9)	1403	(90.7)
Total =	327		1547	

?   You can see that these observed results do agree with what Kettlewell expected to observe based on his research hypothesis. However, might there be some alternative explanation for these data? Provide two additional hypotheses that could explain these same data?

a.

b.

Frequently a single research hypothesis makes multiple predictions. Kettlewell now examined an additional prediction of his original hypothesis and devised an experimental test for it:

If Kettlewell's original research hypothesis is correct,

then (1) we should be able to release known numbers of marked moths of the two morphs in soot-free woodlands and after a period of time collect proportionally more light-colored moths; and (2) we should be able to release known numbers of marked moths of the two morphs in soot-darkened woodlands and after a period of time collect proportionally more dark-colored moths.

In an experimental test of a hypothesis the scientist sets up two groups that differ in a single factor; here, the factor is whether the morphs are light or dark. Kettlewell used paint to mark the moths (on the underside of the abdomen so the paint would not affect the crypticity of the moths) and released them in the woodland areas. Actually, Kettlewell was conducting two complementary experiments, one in each of the two different woodland areas. The data in Table 1.2 shows his results. Were the predictions of his research hypothesis supported?

Table 1.2. Number of light morphs and dark morphs of peppered moth (*Biston betularia*) released and recaptured in soot-free and soot-darkened woodland areas (Kettlewell, 1959).

Morph Type	Soot-Free Woodland		Soot-Darkened Woodland	
	Number Released	*Number (%) Recaptured*	*Number Released*	*Number (%) Recaptured*
Light	393	54 (13.7)	137	18 (13.1)
Dark	406	19 (4.7)	447	123 (27.5)
Total =	799		584	

? Kettlewell was still concerned that some factor other than predation was causing the differences he observed in the two woodland areas. What about alternative explanations for these data? Perhaps the dark moths are more tolerant of pollution than the light moths. Would this hypothesis explain all the data? What if moths move about until they find a woodland area where their crypticity is maximized? Would this possibility explain all the data we have examined so far?

As a final experimental test of his original hypothesis, Kettlewell and his colleagues pinned freshly killed moths of each type on trees in the two woodland areas and observed, while hiding, what happened to them. After many hours of watching, they collected the data in Table 1.3 on birds preying on the moths.

Table 1.3. Number and percent (%) of light and dark morphs of peppered moth (*Biston betularia*) eaten by birds in soot-free and soot-darkened woodland areas. (Kettlewell, 1959.)

Number (%) Eaten by Birds

Morph Type	Soot-Free Woodland		Soot-Darkened Woodland	
Light	26	(13.7)	43	(74.1)
Dark	164	(86.3)	15	(25.9)
Total =	190		58	

? Do these data support Kettlewell's research hypothesis? Do they provide some additional information as well?

To summarize, a research hypothesis is tested by determining if its predictions are true. Some predictions suggest that it should be possible to make certain observations. These predictions lead to an observational test of hypothesis. Because the observational test is nonmanipulative, one can be fairly certain that it represents what happens in nature. Other predictions may suggest that the scientist should be able to make certain events occur. These predictions lead to an experimental test of hypothesis. In an experimental test we manipulate the environment to create two groups that differ in a single factor. This reduces many confounding variables that may be present in an observational test. In general, results from experimental tests are more convincing than results from observational tests of hypotheses. However, because of the manipulations, it is sometimes difficult to be certain that experimental results reflect what happens in nature. In the final experiment, for example, could Kettlewell be certain that the positions of his artificially placed moths were exactly like the positions that free-roaming moths would take?

For many years, Kettlewell's experiments on the peppered moth have served as the textbook example both of the scientific method and of evolution by natural selection (selective predation in this case). Recent criticisms of Kettlewell's work include the fact that the mark-recapture studies in different environments were conducted several years apart and differed in some procedures. And Kettlewell's release of the test moths in unusually large densities, during the day, and onto tree trunks may be less than an ideal experimental design, because peppered moths are active only at night and appear to spend daylight hours high up under tree branches, not on tree trunks. Nevertheless, the Kettlewell experiments remain as a good example of scientific inquiry, a process in which researchers continue to refine the questions they ask and the experimental designs they employ to answer those questions.

ANALYSIS AND INTERPRETATION

A test of hypothesis produces data called the **observed results**. The observed results can take two forms, as expressed by statistical statements called the **null hypothesis**, or H_o, and the **alternative hypothesis**, or H_a. H_o states that the data groups being compared do not differ. H_a states that the data groups being compared do differ. For example, in an experimental test of hypothesis, the null and alternative hypothesis take the following forms,

Null Hypothesis Type
H_o : control data group = experimental data group

Alternative Hypothesis Type
H_a : control data group ≠ experimental data group

The research hypothesis being tested predicts which outcome, H_o or H_a, must be observed to validate the hypothesis. The outcome predicted by the research hypothesis is called the **expected results**. Do the observed results agree with the expected results or do they differ? We need to answer this question in an <u>objective</u> way in order to determine if our hypothesis is valid. For example, we see in Table 1.1 that in the soot-free woodland, 324 moths were light morphs and only 3 were dark morphs. If there had been no difference in the numbers of the two morphs captured (as stated by H_o), we would have expected half to be of one form and half of the other, or approximately 163.5 of each morph. Are the numbers Kettlewell observed, 324:3, sufficiently different from 163.5:163.5 for us to be certain that our hypothesis is valid? In this study the differences are large enough so that additional data analysis may not be needed. This is often *not* the case in biology.

Data analysis usually includes some type of **statistical testing** that compares the data in an objective manner and allows the scientist to calculate a probability value that reflects how certain he or she is in concluding that the original research hypothesis is valid. In any test of hypothesis the sample size (in this case, the number of moths captured) is an important consideration in determining our level of confidence in the results. Statistical tests take sample sizes into account in comparing the data groups.

If the results of data analysis allow the scientist to consider the hypothesis valid (that is, verified by the data), then it becomes part of the explanatory system and can be used to interpret new observations. Thus, new scientific knowledge builds on what is already known. However, if the results of the study do not verify the hypothesis, it is rejected and additional, more accurate observations may be necessary to formulate a new hypothesis. Even if the new observations are accurate, our reasoning in constructing the hypothesis may be at fault. Are there other explanations for the observations? We may need to examine our explanatory system. Perhaps our observations are good but our research hypothesis is invalid because it is based on a faulty explanatory system that needs to be modified based on our new observations. Thus, scientific knowledge is constantly changing and being updated. This discussion of the scientific method is summarized in Figure 1.1.
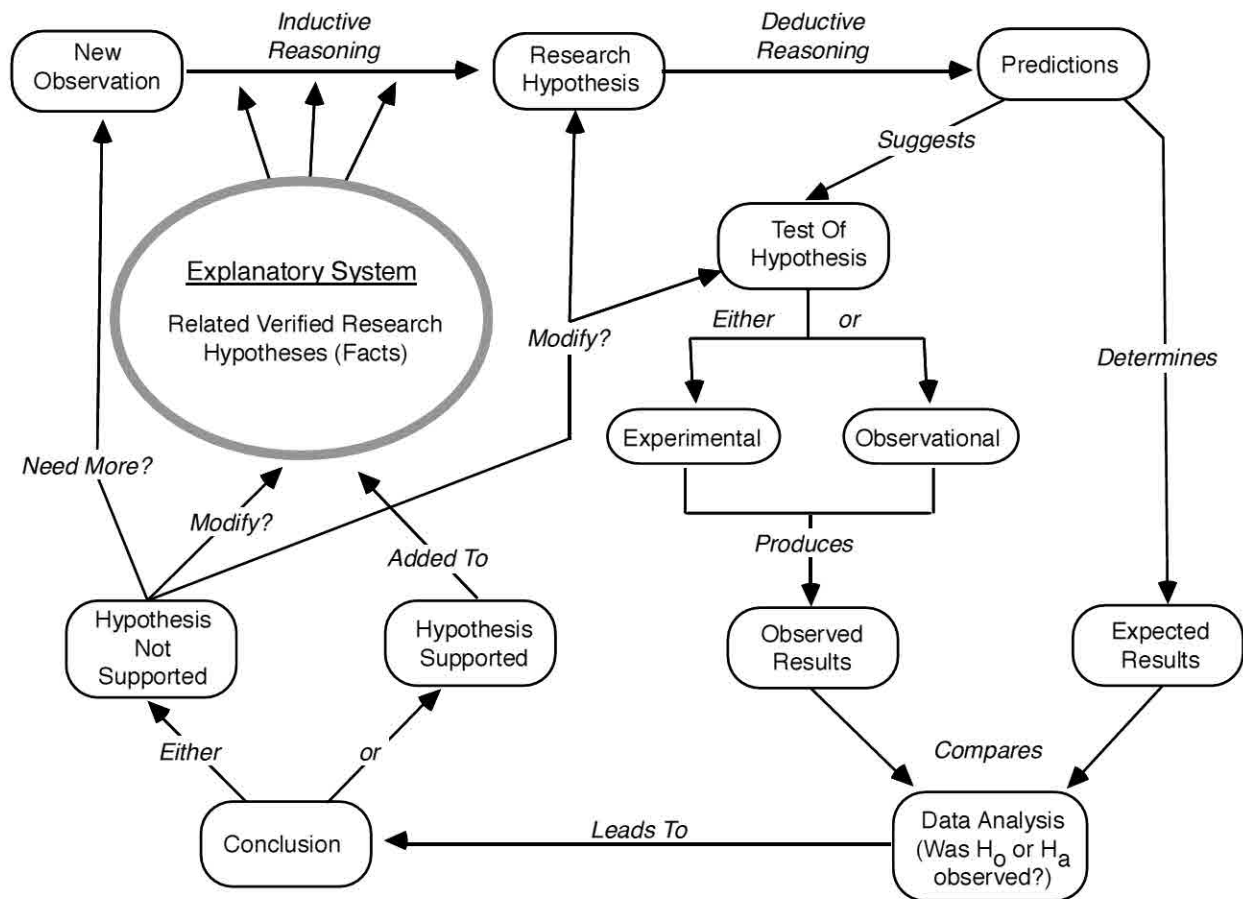
Figure 1.1. Schema showing the cycle of activities and reasoning in science.

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Revised May 2010
Mark A. Sarvary

Biology as a Science Worksheet

NAME: _____   **TA:**_____

1. What was the new observation that Kettlewell wished to explain?

2. What were some of the established "facts" in the explanatory system used by Kettlewell to interpret this new observation?

3. What is a research hypothesis? Give an example of a research hypothesis formulated by Kettlewell in his study of the Peppered Moth.

4. What are the strengths and weaknesses of an experimental test of hypothesis?

5. What are the strengths and weaknesses of an observational test of hypothesis?

CHAPTER 2 – MICROSCOPY, CELLS AND TISSUES

LABORATORY SYNOPSIS

In this laboratory you will learn the fundamental principles and procedures for light microscopy so that you can use it efficiently in subsequent laboratories. The emphasis in this laboratory is on microscopy. The biological materials you will use have been selected to demonstrate certain principles of light and electron microscopy and to supplement your understanding of cell structure and organization.

During this laboratory you should obtain an understanding of the basic principles of light and the electron microscopy and preparation of biological materials for microscopic examination. By using and comparing the high magnification compound and stereoscopic microscopes you will become aware of their similarities and differences. You will learn how to use the vernier on the mechanical stage of a compound microscope to measure microscopic and small macroscopic objects. The technique of dark field illumination in light microscopy will be demonstrated and you will use a simplified type of polarization light microscopy. Your observations of cells and tissues will enable you to understand the advantages and limitations of compound and stereoscopic light microscopy and transmission and scanning electron microscopy.

LEARNING OBJECTIVES

Conceptual

At the end of this laboratory sequence you should

1. understand the concepts of magnification, contrast and resolving power with respect to microscopes.

2. understand generally why the resolving power of an electron microscope is much better than that of a light microscope.

3. know the difference between transmission and scanning electron microscopy with respect to the use of electrons and the preparation of biological material for microscopic study.

4. know the fundamental differences between the compound and stereoscopic microscopes with respect to magnifying ability, resolving power, orientation of the observed image, preparation of biological material for microscopic study, and use of light.

5. understand the relationship between magnification and size of the field of view in light microscopy.

6. know the units of measurement used in microscopy, how they are related and how to convert one unit to another.

7. understand the rationale for the basic histological procedures of cutting thin sections of biological material and staining them.

8. have an appreciation for the use of histochemical techniques for showing selected components of cells and tissues.

9. understand the basic difference between bright field and dark field microscopy with respect to the use of light to form an image.

10. know what characteristics of cellular and tissue components are revealed by polarization microscopy.

11. have an appreciation for the use and limitations of light microscopy, scanning electron microscopy and transmission electron microscopy in the study of cell and tissue structure.

Methodological

At the end of this laboratory exercise you should

1. know how to properly use the compound and stereoscopic microscopes. You will learn the correct methods of focusing the microscope and how to adjust light intensity for the best viewing. You will be able to determine the total magnification of an image and estimate the sizes of objects viewed in the microscope. You will learn how to take care of these microscopes, to clean the lenses and to store them properly after use.

2. be able to identify from a photo/micrograph the type of microscopy used to obtain the image.

READING ASSIGNMENT

In addition to reading this chapter before the lab, you should read Appendix 1 to review the metric system. You should also read the following section in Campbell and Reece, *Biology*, 8th ed. (2008):

A TOUR OF THE CELL: p. 94-97 (Concept 6.1)

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

1. What is the resolving power of a lens system?

2. What are the functions of the objective and ocular lenses of a compound microscope?

3. Assume you are using a compound microscope with a 40X objective and a 10X ocular, what is the total magnification of this lens system?.

4. Why are the resolving powers of electron microscopes much smaller than those of light microscopes?

5. Why must biological materials be in very thin sections to be seen using a high magnification compound light microscope or a transmission electron microscope?

6. Why must most thin sections of biological materials be stained (dyed) to be seen using a compound light microscope or a transmission electron microscope?

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INTRODUCTION

If a single instrument has given biology the greatest advances, it has been the light microscope. The light refers to the use of visible wavelengths of radiation (light) in this instrument to form an image of the object observed. There are several methods of light microscopy that differ mainly in how light is used. Prior to the rapid development and widespread use of electron microscopes, which began around 1960, most of the information regarding the structural organization of cells and tissues was obtained by light microscopy. Furthermore, much of our information about the physiology of cells and tissues was, and is, obtained from various light microscopic methods. Although electron microscopy gives us a closer and/or different view of cells and tissues than that obtained with light microscopy, the latter allows us to observe living cells, which generally cannot be done using electron microscopy. Modern cytological and histological research depends greatly on both light and electron microscopy. Light microscopy is used in all areas of biological study, including biochemistry and ecology, and its technology is still developing.

You will be using light microscopes in several of your laboratory exercises in this course; therefore, it is important for you to know how to use these instruments properly so you can obtain maximum benefit from using them and avoid inadvertently damaging them. You will not use electron microscopy in this course; however, as a biology student you will see many micrographs obtained by this technology, and a general understanding of it will be helpful in your interpretation of electron micrographs. Therefore, a brief comparison of light and electron microscopy has been included in the background information for this chapter.

THE PRINCIPLES OF MICROSCOPY

The physics of microscopy is beyond the scope of this course, but an elementary knowledge of its principles is necessary for understanding the limitations and possible uses of various kinds of microscopy. A microscope produces a magnified image of an object. A light microscope uses glass lenses and visible radiation while an electron microscope uses electromagnetic or electrostatic lenses and an electron beam. The image observed is produced by the light or electrons interacting with the object.

General description of light microscopes

Figure 2.1 is a diagram of a high-magnification compound microscope. A compound microscope has two or more lenses. One lens, called the **objective**, produces a magnified image of the object. Another lens, the **ocular** or **eyepiece**, magnifies the image produced by the objective. These two lenses are situated at opposite ends of the microscope's **body tube**. The total magnification of a given compound lens system is the product of the magnification of the ocular (usually 10X) and the magnification of the objective, which can be varied. Focusing a microscope is accomplished by changing the distance between the objective and the object observed, which is supported on a platform called the **stage**. Two types of microscopes are available for your use in the laboratory:

1. A high-magnification microscope, generally called a **compound microscope**, contains a single compound lens system. This microscope provides total magnifications of 40X, 100X and 400X. Most of our compound microscopes have a single ocular (or eyepiece) lens and are called **monoculars**; however, each lab room has a few binocular compound microscopes. The use of two ocular lenses reduces eyestrain, but does not provide stereoscopic vision, because only one image is formed by the single objective.

2. A stereoscopic binocular microscope, also called a **dissecting microscope**, has two complete compound lens systems and gives total magnifications from 8X to 50X. This type of microscope has a compound lens system for each eye and gives the user a true stereoscopic image, because the two objectives produce images from different positions.

Technically, both of these types of microscopes are compound microscopes in that they contain objective-ocular lens systems; however, the name **compound microscope** is usually applied only to high-magnification microscopes such as that shown in Figure 2.1. Our laboratories are equipped with compound microscopes whose designs vary somewhat from that shown in Figure 2.1. For example, focus adjustment knobs are at different locations on different models. However, the same optical principles apply to all types of compound microscopes.

One of the major differences between the compound microscope and the stereoscopic dissecting microscope is the method of illuminating the observed object. The distance between the objective lens of the compound microscope and the observed object is so small (especially when the high magnification objective is used) that the object cannot be sufficiently illuminated from above the stage and must be illuminated from below. Light first passes through the **substage condenser**, a lens which focuses light on the object lying on the stage (see Figure 2.1). Then the light passes through the object and into the microscope's lens system. Since the compound microscope utilizes light <u>transmitted</u> by the object the material being examined must be transparent, but components of the material should be differentially transparent so that contrasts can be seen. Substances vary with respect to the quantity and wavelengths of light that they absorb. The more light an object absorbs, the darker it appears. The color that an object appears to our eyes depends on the wavelengths of light that the object transmits or reflects. Unfortunately, most cellular components are fairly transparent, but histologists and cytologists have developed techniques for increasing the contrast of cellular components by staining them with pigments of various colors. You will use some of these techniques in subsequent laboratories.

The stereoscopic dissecting microscope lacks the components that are beneath the stage of the compound microscope (e.g., condenser, diaphragm, lamp). The optical principles of microscopy explains why. An object observed with a stereoscopic microscope is generally illuminated from above the stage. The relatively large distance between the objective and the object allows sufficient illumination from above so that the objective can collect light reflected, diffracted, or refracted by the object to form an image.

Magnification and Resolving Power

Although a microscope is a magnifying instrument, magnification alone does not necessarily improve one's ability to observe details. The combination of a microscope's magnification and resolving power enables one to distinguish between two small adjacent objects. The **resolving power** of a lens system is its ability to make closely adjacent objects appear distinct, and is expressed as the <u>minimal distance</u> between the objects when they are still distinguishable from each other. The resolving power of a lens system depends on the wavelength of radiation used, the refractive index of the medium in the space between the object and the objective lens, and the acceptance angle of the objective lens. The mathematical relationships among these parameters are given by equation 1:

$$\text{resolving power} = \frac{0.6\lambda}{n\sin\alpha} = \frac{0.6\lambda}{\text{N.A.}} \qquad (1)$$

λ = wavelength of the radiation

n = refractive index of medium between objective and object (n = 1 for air)

α = one half the acceptance angle of the objective (see Figure 2.2)

N.A. = numerical aperture ($n\sin\alpha$)

When light is reflected from or transmitted by an object on the microscope stage, its rays diverge. A solid cone of light rays coming from the object is accepted by the circular objective lens (see Figure 2.2). In three dimensions, the objective lens is a plano-convex disc (one side is flat, the other is convex). Its diameter is the diameter of the base of the cone of light rays coming from the object. The **acceptance angle** of the objective is the angle subtended by the apex of the cone. The acceptance angle depends on the diameter of the objective and the distance between the objective and the object.

Ocular
eyepiece

Upper part
of body tube

Rotatable nosepiece

Arm

Objective (high power)

Objective (low power)

Object to be observed

Mechanical
Stage

Stage

Substage
condenser
adjustment knob

Substage condenser
housing

Fine focus
adjustment knob

Arm to regulate
iris diaphragm

Coarse focus
adjustment knob

Lamp housing

Base
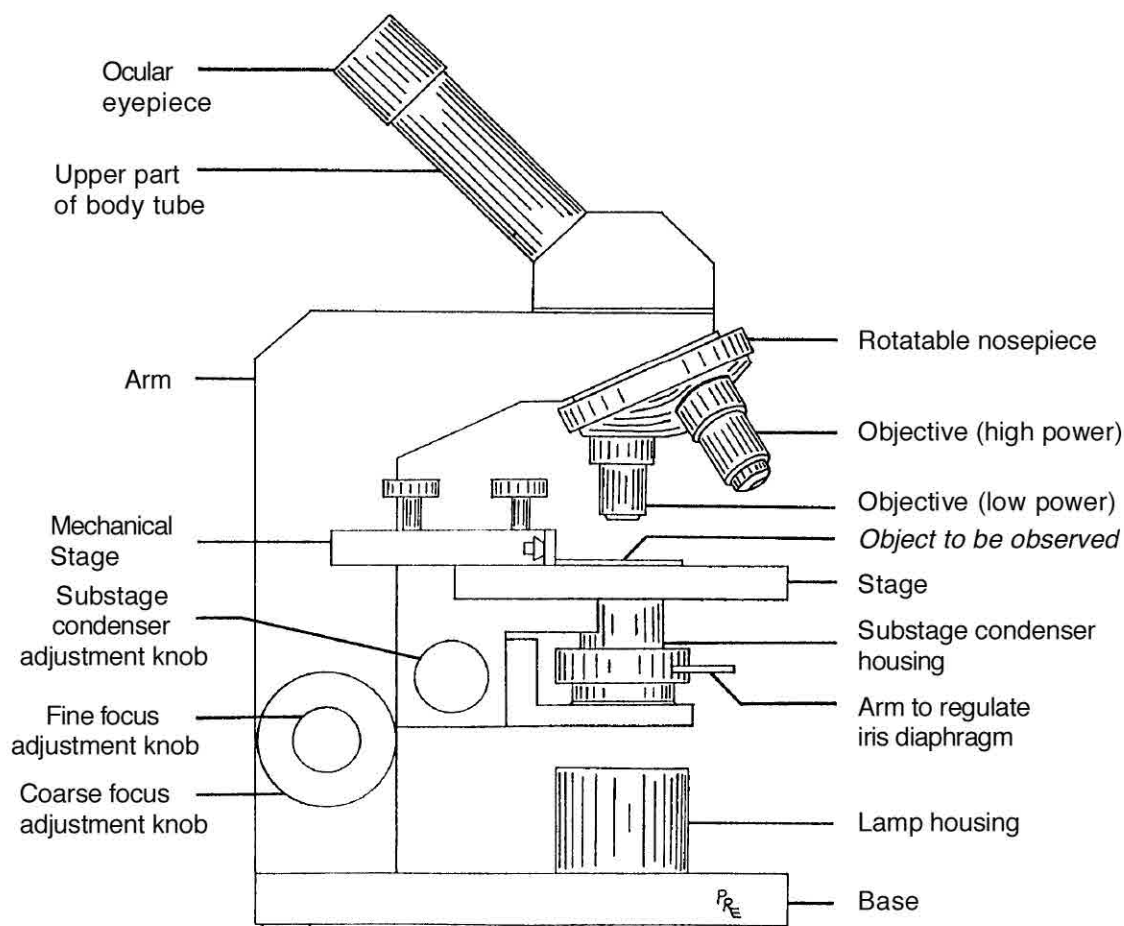
Figure 2.1. Diagram of a compound microscope showing the principal parts.

Figure 2.2 Diagram showing a two dimensional view through the middle of the objective lens and the cone of light rays accepted by it. α is the angle formed at the vertex of the side of the cone and a line perpendicular to the center of the objective; it is half the acceptance angle of the objective.

microscope
objective

α

acceptance angle
of objective
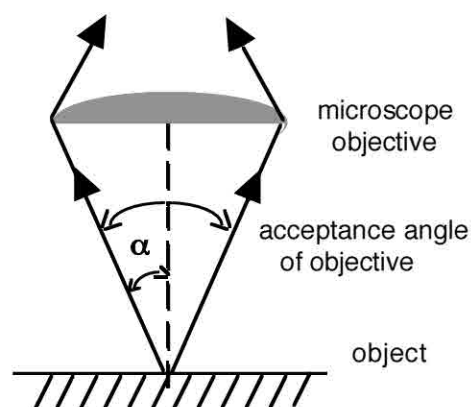
object

The denominator of equation 1, $n \sin \alpha$, is called the **numerical aperture** of the objective lens and usually is engraved on the tube holding the lens. The resolving power of a lens can be improved (made smaller) by decreasing the wavelength of the radiation used and/or increasing the numerical aperture of the objective. If air is the medium between the objective lens and the object, $n = 1$ and the numerical aperture can be increased only by increasing α. Notice in Figure 2.2 that α increases as the distance between the objective and the object decreases. The maximum value for α is a little less than 90°; consequently, the upper limit for $\sin \alpha$ is slightly less than 1. Therefore, with air between the objective and the object, the numerical aperture is always less than 1. A numerical aperture greater than 1 can be

obtained by using a very high magnification (100X) objective called an oil immersion objective and a special immersion oil between the objective and object. When an oil immersion objective is used, it is so close to the object that sin α is greater than 0.9. The refractive index, *n*, of the oil is 1.4. Thus, the numerical aperture is about 1.3. From equation 1 we see that

resolving power of an oil immersion objective with immersion oil = $\dfrac{0.6 \lambda}{1.3}$ $\cong$ 0.5 λ

By maximizing the numerical aperture of the objective, one can achieve a resolving power equal to about one-half the wavelength of the radiation used. In light microscopy the image produced is generally recorded directly by the human eye whose perception of radiation is limited to a small range of wavelengths. Consequently, the human eye limits the practical resolving power of a light microscope. The range of radiation wavelengths perceived by the human eye is from 400 nm to 700 nm; optimal perception is near 550 nm (see Appendix I: THE METRIC SYSTEM). Wavelengths shorter than 450 nm are not perceived strongly enough to be useful in light microscopy; therefore, the resolving power of the light microscope using radiation perceived by the human eye is limited to 0.5 " (400 nm) = 200 nm or approximately 0.2 μm (see Appendix I: THE METRIC SYSTEM).

The human eye has a resolving power of about 0.1 mm (= 100 μm), which is determined by the limited size and arrangement of light sensitive cells composing the retina. When images from two separate objects fall on the same light-sensing cell, the two images are perceived as one. In our calculations above, we determined that an oil immersion objective with immersion oil could resolve, in the image formed, two minute objects only 0.2 μm apart. However, the human eye cannot distinguish objects that are closer than 100 μm apart. Thus, for the eye to be able to see that the two objects are distinct in the image, the image must be magnified enough to make the distance between the two objects at least 100 μm. What magnification is required?

That is: 0.2 μm " magnification = 100 μm (resolving power of eye)

Solving the above equation gives:

Magnification = $\dfrac{100 \ \mu m}{0.2 \ \mu m}$ = 500 X

The 100X magnification of the objective lens alone is not sufficient, and an additional lens, the ocular (or eyepiece), is used for further magnification (see Figure 2.1). The **total magnification** of a compound microscope is the product of the magnifying powers of the objective and ocular. The magnifying power of an ocular is 10X or 15X (our microscopes have 10X oculars). The total magnification of a compound microscope with a 100X oil immersion objective and a 10X ocular is 1000X, more than enough to enable the human eye to see the minimal distance resolvable by the objective. The function of the ocular is only magnification; it cannot improve the resolving power of the microscope.

The major factor limiting the resolving power of the light microscope is the wavelength of radiation used. Electron microscopes have much smaller resolving powers because the electrons they use have very much shorter wavelengths. The source of radiation in an electron microscope is an electron gun. Voltage applied between components of the gun accelerates electrons as they are emitted. The wavelength of an electron is inversely related to the square root of the accelerating voltage. Most electron microscopes use voltages between 40,000 and 100,000 volts (or 40-100 kV; kV is the abbreviation for kilovolt; 1 kV=1000 volts). An electron accelerated by 100 kV has a wavelength of 0.0037 nm, which is approximately 1/100,000 the shortest wavelength of visible light. If an electron objective lens with a numerical aperture of 1 were available, then a 100 kV electron microscope could have a resolving power of 0.0022 nm (from equation 1). This distance is about 1/100 the diameter of an atom! However, the

objective lens of an electron microscope requires a very narrow angular aperture, resulting in a numerical aperture of 10^{-2} to 10^{-3}. Using equation 1 with 10^{-2} as the numerical aperture ($n \sin \alpha$), the resolving power of a 100 kV electron microscope is about 0.2 nm. Somewhat smaller resolving powers can be obtained with higher electron accelerating voltages, but the high energy of the electrons may damage the specimen.

Major Methods of Electron Microscopy

Electron microscopes use electromagnetic and electrostatic lenses to focus the electron beam and form magnified images. Since the human eye cannot see electrons, the final image is projected onto a special viewing screen and/or onto a photographic plate, if a permanent record of the image is desired.

Transmission electron microscopy (TEM) is similar to transmission light microscopy in that it depends on the differential transmission of electrons through the object. As on the compound light microscope, the object lies between the condenser lens and the objective lens which have the same names and functions. The lens in the transmission electron microscope which corresponds to the ocular of the compound microscope is called the projector lens. It magnifies the image made by the objective and projects the enlarged image onto the viewing screen. TEM requires extremely thin sections of the specimen (approximately 1/100 the thickness of sections used for transmission light microscopy). A two-dimensional image of the specimen is obtained. Since very thin sections of biological materials are essentially transparent to electrons, components of the sections are differentially stained with heavy metal ions (usually osmium tetroxide or lead or uranium ions) to increase their electron scattering ability, and thus increase contrast in the image. The outstanding features of transmission electron microscopy are high magnification and resolution. With the best transmission electron microscopes individual molecules can be distinguished in the image (see Table 2.1).

Scanning electron microscopy (SEM) uses an electron gun and a condenser lens system to produce a finely focused primary electron beam on the specimen. Unlike TEM, there are no lenses on the other side of the specimen because SEM does not use electrons transmitted by it. Most of the scanning electron micrographs seen in your text book and reference books have been obtained by the emissive mode of SEM. When electrons strike the specimen, secondary electrons of much lower energy are emitted from its surface; furthermore, some of the electrons from the primary beam are reflected (or back scattered) by the specimen's surface. The intensity of emission of secondary and back scattered electrons depends on the angle at which the primary electron beam strikes the surface, which in turn, depends on the specimen's topographical features. Often it is necessary to coat the specimen's surface with a metal (usually gold, platinum or a gold/palladium alloy) to enhance the emission of electrons. As the primary electron beam scans the surface of the specimen (much like the picture tube of a television), the electrons emitted from the surface are collected and their signals are amplified and converted into a visual image of the surface. The outstanding features of emissive scanning electron microscopy are the depth of field obtained and the three-dimensional quality of the micrographs. Since scanning electron microscopy does not depend on the passage of electrons through the specimen, the thickness of the specimen is not critical.

Table 2.1. A comparison of important features in light- and electron microscopy.

Type of microscopy	Minimum resolving power	Maximum effective magnification	Appropriate thickness of specimen
Transmission light	200-300 nm[1]	1200X	5-15 μm[1]
Transmission electron	0.2-0.5 nm	1,000,000X	5-100 nm
Scanning electron	5-20 nm	200,000X	not critical

[1] See Appendix 1. "The Metric System"

PREPARATION OF BIOLOGICAL MATERIAL FOR MICROSCOPY

Live cells and tissues in sufficiently thin specimens can be observed with a high magnification light microscope, but many of their details are invisible because they lack contrast. To obtain and stain the thin sections of material required for transmission light and electron microscopy and to coat the surfaces of specimens used in SEM, the biological material must be killed and preserved.

In the case of relatively large multicellular organisms, the desired organ or tissue is removed from the organism and immediately placed in an aqueous fixing solution which kills and preserves the cells. Components of the fixing solution bind to lipids, proteins, and nucleic acids, crossing-linking them and fixing their positions in the cells.

To facilitate cutting the thin and ultra thin tissue **sections** required for transmission light and electron microscopy, and to maintain the proper spatial arrangement of cells in the tissue during the cutting of sections the tissue is embedded in a solid medium. Paraffin wax is the usual embedding medium for cutting thin sections for transmission light microscopy, but it is inappropriate for cutting ultra thin sections needed for TEM (see Table 2.1). Plastics are used to embed specimens to be cut for TEM. The aqueous solution in the tissue is replaced (dehydration) with liquid embedding medium which then solidifies (embedding). A block of solid embedding medium containing the specimen is mounted on a special cutting instrument called a **microtome**. Sections of uniform thickness are cut from the tissue-containing block and mounted on the appropriate surface; a microscope slide for light microscopy and a specimen support grid (usually made of copper) for TEM.

Sections of structures prepared for microscopy are named according to the plane in which they are cut. Commercially prepared slides with sections have labels indicating, by an abbreviation, the plane of the cut used to make the section. The names, abbreviations and definitions of the most common sections are listed in Table 2.2. See Figure 2.3 for examples of sectioning planes and sections.

Table 2.2. Section names, abbreviations and definitions.

Name	Abbreviation	Definition
Transverse- or Cross-section	t., t.s., c., c.s., or x.c.	Cut perpendicular to the longitudinal axis of the structure.
Longitudinal section	l. or l.s.	Cut parallel to the longitudinal axis of the structure.
Median section	med. or m.	Cut through the middle of the structure.

Combinations of abbreviations may be used. For example, m.l. is a median longitudinal section.

Most of the components of cells are nearly transparent and have poor electron scattering ability; consequently, they are not readily seen using light microscopy or TEM unless they are stained to increase contrast. Most of the stains used for light microscopy are colored dyes whose molecules bind to certain cellular components. Since the dyes are in aqueous solution and paraffin is impervious to water, the paraffin is removed from the tissue, which is then rehydrated enabling the aqueous dye solution to penetrate the cells. Following the staining procedure water is again removed from the tissue, which is then mounted in a special transparent mounting medium and covered with a cover slip. Contrast in the stained specimen is the result of differential absorption of light.

Stains for ultra thin sections used in TEM are heavy metal ions such as osmium tetroxide (OsO_4) and the salts of heavy metals such as uranyl acetate and lead citrate. The heavy metal ions bind to certain cellular components and increase the electron scattering ability of these components. Contrast in the image formed is the result of differential scattering of electrons by various parts of the cells.

Most of biologists' understanding of internal cell structure has come from TEM because of its better resolution of the cell's fine details. SEM has been used mainly to study the outer surface details of cells. However, there are techniques which enable one to observe the internal structure of cells using SEM. **Freeze-fracture** involves killing and fixing the tissue in a fixing solution, freezing the tissue in liquid nitrogen so it is very hard and brittle, cracking open some of the brittle cells, thawing and drying the tissue, and coating the exposed internal structures of the cracked cells with a thin layer of platinum or gold.

It is important to realize that the stained cells and tissues which you observe on microscope slides and in photo-and electron micrographs are not the same as living tissues. In general, the shape and gross structure of the cells have been altered little in the preparation, but various fluids of the living cells have been removed or solidified. The normally fluid, dynamic systems such as cell membranes have become fixed. The colors of various cellular and tissue components are not natural, but are the result of staining procedures. Biological specimens that have been prepared for microscopic study may contain artifacts of the procedures used to prepare them for microscopy. The appearance of artifacts depends on the methods of fixation, dehydration, embedding, staining, and sectioning, and the skill of the preparator.

Sectioning Plane	Transverse or Cross	Nonmedian Longitudinal	Median Longitudinal
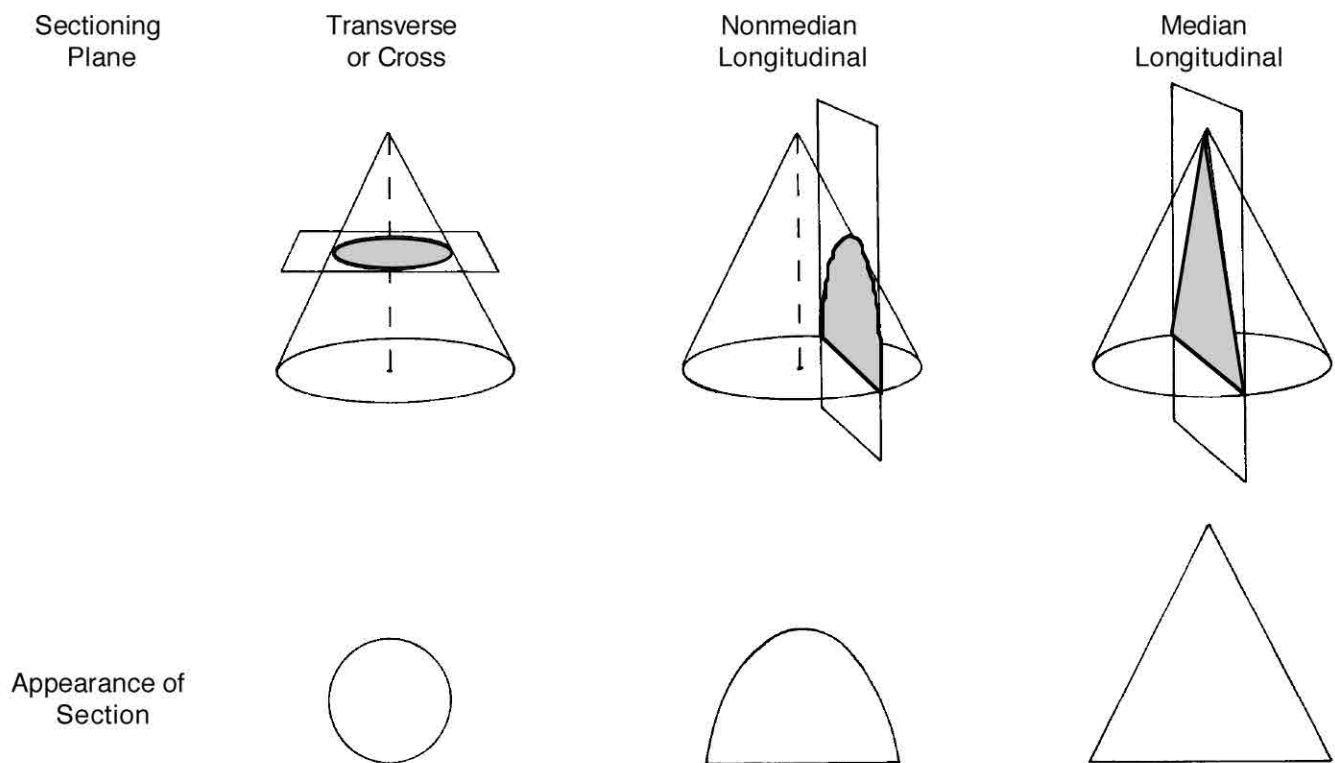
Figure 2.3. Diagrams of cones showing planes for cutting sections and the shapes of the sections. The longitudinal axis of each cone is shown by a broken line.

LABORATORY EXERCISES

USE AND COMPARISON OF COMPOUND AND STEREOSCOPIC MICROSCOPES

Your lab instructor will ask you to obtain and sign out a compound and a stereoscopic microscope from the microscope cabinets. When carrying either microscope, <u>use both hands</u>--one under the base and the other to grip the body arm.

The Compound Microscope: Components And Their Functions

Before you use this microscope, become familiar with it by identifying the following components. Use Figure 2.1 for reference and, if necessary, ask your lab instructor for assistance.

An **ocular** (or **eyepiece**) lens (generally providing 10X magnification) is located at the upper end of a 160-mm **body tube**. At the other end of the tube is a **rotatable nosepiece** which bears three **objective lenses** having different magnifying capabilities. The magnifying power of each objective (4, 10 and 40) is engraved on its side.

An object to be examined is placed on the **stage** over the **stage opening** and is illuminated from below. Usually specimens are mounted on microscope slides for observation. Your microscope has a **mechanical stage** with a spring clip and frame to hold the microscope slide. Movement of the slide holder along x and y axes is regulated by turning the two knobs on the side of the mechanical stage.

The **substage condenser** is a circular glass lens mounted below the stage opening. It can be moved up and down by moving an arm or rotating a knob beneath the stage (the method depends on the microscope model). The substage condenser should be in its <u>uppermost position</u> for most routine microscopy.

The **iris diaphragm** is mounted immediately below the substage condenser in the same movable frame. If you are using a black microscope locate the **arm** used to regulate the iris diaphragm. The iris diaphragm on a beige microscope is regulated by turning a knurled **ring** around it.

The **lamp housing** is mounted on the base below the stage opening. An off/on switch and light intensity control knob is located on the right side of the base of a beige microscope. A black microscope has an off/on switch for the lamp on the electrical cord; no electrical control of light intensity is available on this microscope.

Adjusting the size of the opening formed by the iris diaphragm changes the acceptance angle of the microscope (Figure 2.2). Closing it makes the acceptance angle smaller, opening it makes it larger. This alters the resolution of the microscope, but it also alters the **contrast**. The best trade-off between resolution and contrast must be found for each objective lens. Normally to get high resolution you want the contrast to be as low as possible, and so the iris diaphragm should be wide open. But some contrast is needed to see the object. Especially when first searching for small and obscure items it is useful to have high contrast; by closing down the iris diaphragm. Also notice that closing down the iris diaphragm does not change the intensity of the light hitting the specimen, but it does alter the contrast. Adjust the lamp voltage regulator to change the light intensity.

Focusing the image is accomplished by moving the stage, and hence the specimen, toward or away from the lens system. **Coarse** and **fine focus adjustment knobs** are used for this purpose. Locate these knobs. The larger knob is for coarse adjustments. The fine focus knob is on the outside of the coarse focus knob.

In many microscopes, you may see a black line partially across part of the field of view. This is a pointer, mounted in the ocular, which enables you to point out particular objects or regions of the material under observation. A few of you may have an ocular with a pointer.

Procedures in Using the Compound Microscope

1. Clean the ocular, objectives, and substage condenser with the special **lens paper** provided. (NOTE: Nothing but **lens paper** should be used on the lenses; anything else may scratch them.)

2. Set the substage condenser in its uppermost position.

3.   Rotate the nosepiece so that the lowest magnification (4X) objective is above the stage opening; lower the stage, if necessary, to prevent the objective from touching it.

4.   Use the course focus knob to raise the stage until it is as high as it can go. <u>Note</u>: These microscopes are built so that with the 4X objective in place the objective will not hit the specimen on the stage.

5.   Turn on the microscope lamp. Look through the ocular (eyepiece) and open the iris diaphragm fully to give maximum diameter to the circular field observed.

6.   Remove the ocular lens from the body tube. Look into the body tube and observe the brightly illuminated circle of the back of the objective lens. Close the iris diaphragm until the diameter of the illuminated circle is about 3/4 of its initial diameter. Replace the ocular lens. This procedure gives you the best resolution of the image observed with either the 10X or the 40X objective. Pay attention to the relationship between contrast and resolution.

?   What is the total magnification of your microscope when you are using the 4X objective? _____

7.   Obtain a microscope slide with printed letters (Cornell) mounted on it. Notice that the letters are covered with a small thin piece of glass called a **coverslip**. Open the clip of the mechanical stage and place the slide <u>with the coverslip on the top side</u> in the slide holder.

       Caution: If the clip has a strong spring, do not allow it to swing freely back to the slide. Some clips move with enough force to break slides.

8.   Using the knobs on the mechanical stage, move the slide so that some of the letters are directly over the center of the substage condenser.

9.   While looking through the ocular, slowly lower the stage by turning the coarse adjustment knob until a letter comes into focus. Obtain a sharp focus by using the fine adjustment knob.

10.   If necessary to obtain a clear view of the specimen, adjust the light intensity using the variable light intensity control knob (beige microscopes only) to give a light gray bright field.

11.   Use a ruler for reference and visually estimate the distance, in mm, between the objective and the object when the image is in focus. <u>Note</u>: a precise measurement is not necessary. This distance is called the **working distance** of the objective. Record this estimate for future reference.

       Working distance of the 4X objective:_____.

12.   Move the slide so that the letter "e" is centered in the field of view. Notice that the image of the letter appears inverted (upside down and backwards) compared to the orientation of the letter on the stage. Move the slide from right to left. Which way does the image appear to move? These inversions are caused by the optics of the microscope. You will probably require some practice in compensating for these optical inversions.

13.   When you want to see more detail, it is necessary to use a higher resolving objective, but you should always locate the specimen by <u>first</u> using the low magnification objective. After you have focused the part of the specimen in which you are most interested and centered it in the viewing field, switch to a higher magnification as described in the next step.

14.   While watching from the side of the microscope, rotate the 10X objective into position above the stage opening. It should not be necessary to adjust the distance between the stage and the body tube before making this change. Most of the microscopes have **parfocal objectives**; consequently, if the image was in focus with the 4X objective, it should be nearly in focus when you change to the 10X objective, and only a minor adjustment with the **fine focus knob** should be required to complete the focusing. In viewing the field at 100X magnification, two things should be apparent: both the light intensity and the portion of the specimen visible within the microscope's field of view are much less with 100X magnification than with 40X magnification.

15.   Increase the light intensity on the specimen by adjusting the variable light intensity control knob.

16.   Estimate the distance, in mm, between the objective and the object when the image is in focus, and record this estimation.

        Working distance of the 10X objective: _____

17.   After you have focused the part of the specimen you are most interested in and centered it in the field of view, rotate the 40X objective into place. Note: Remember to watch from the side while you rotate the objective into place. Make any focusing adjustments with the **fine focus knob**.

18.   You will again notice that the light intensity and the portion of the specimen visible within the field of view have diminished. Increase the light intensity to an optimum level.

19.   Estimate the distance, in mm, between the objective and the object when the image is in focus, and record this estimation.

        Working distance of the 40X objective: _____

?   What is the relationship between magnifying power and working distance?

        How do you expect focal distance to alter the resolution between two lens of the same magnification?

❑   Note that each objective has a decimal fraction engraved on its side; the 40X objective has two. This decimal fraction (the larger of the two on the 40X objective) is the **numerical aperture** (N.A.) of the objective. Record the N.A.s of the objectives.

            N.A. of 4X objective: _____

            N.A. of 10X objective: _____

            N.A. of 40X objective: _____

        The sequence of steps you have just used should be employed whenever you view a specimen with the compound microscope. Page 37 is a reference sheet for using the compound microscope. When you complete it, it will have information useful to you whenever you use a compound microscope.

        In some of your laboratory sessions you must use the microscope for an extended period of time. To avoid fatigue, you should learn to view with a microscope keeping both eyes open. To master this technique, imagine yourself staring at an image some distance below the level of the table as you peer into the eyepiece. The sooner you have learned this technique, the more likely you are to avoid a headache and fatigue.

The Stereoscopic Binocular Microscope (Dissecting Microscope)

This type of microscope consists of two compound lens systems which are focused on the same region of an object, but each eye views the field from a different angle. This arrangement provides the microscopist with binocular vision and increased depth perception. This same principle is used to obtain 3-D images of topographical and manmade features on the earth's surface from aerial photographs.

The optical systems of stereoscopic microscopes include prisms to eliminate the image inversion which is a characteristic of compound microscopes. The relatively large depth of field and the uninverted image obtainable with the stereoscopic microscope make it particularly useful for microdissection and manipulation of small objects; thus, it is often called a **dissecting microscope**. Most stereoscopic microscopes feature variable or zoom magnification. This is accomplished by varying the distance between the objective and ocular lenses, since magnification is directly proportional to the body tube length. As with the high magnification compound microscope, total magnification of the image is calculated by multiplying the separate magnifications of objective and ocular lenses. Most of our microscopes are equipped with 10X oculars.

Varying the magnification is accomplished by:

Rotating a knob on either side of the body tube of a beige microscope.

Rotating a ring around the center of the body tube of a gray microscope.

?   The magnifying powers of the **objectives** are engraved on the rotatable structure. What is the range of magnification possible with your instrument?

_____

The illumination source for your microscope is an attached lamp requiring a separate transformer to regulate light intensity. Transformers are kept in the top drawers of the lab benches.

The lamp should be positioned so that light reflects off the specimen and then enters the lens system. <u>Reflected light</u> is better for color and depth perception than is the <u>transmitted light</u> used with the compound microscope.

Procedures in Using the Stereoscopic Binocular Microscope

Practice using the stereoscopic microscope with the word slide used previously.

1. Usually the viewing plate on the microscope's stage has a black side and a white side. Select the side that gives the greatest contrast between the specimen and the background. You may have to loosen a screw which holds the plate. <u>Note</u>: Some microscopes have a glass plate. Be careful when you carry your microscopes around. The glass plate may slip out and break.

2. Place the specimen on the viewing plate, set the microscope at the lowest possible magnification, and correctly position the light source.

3. Adjust the distance between the two oculars to match the distance between your own pupils.

4. Note that the focus of either of the oculars can be adjusted to compensate for vision differences between your two eyes. Look through one ocular first, with one eye closed, and, using the focus knob, clearly focus the image. Now, look through the other ocular with the other eye closed, and rotate the ocular's milled cuff until you obtain a clear focus. Do the letters appear inverted?

5. Vary the magnification. Does the image remain in focus as you change magnification?

Measuring Objects with a Microscope.

Frequently a biologist needs to know the dimensions of the object s/he is examining with a microscope. The approximate size of an object can be estimated by comparing its dimensions with the diameter of the viewing field. To do this it is necessary first to measure the diameter of the field of view.

Measuring the Field Diameter of a Dissecting Microscope.

1. Place the centimeter scale of a small ruler on the stage of your dissecting microscope and measure, in mm, the diameter of the field at 10X, 20X, and 30X magnification.

    Field diameter at 10X magnification = _____

    Field diameter at 20X magnification = _____

    Field diameter at 30X magnification = _____

?   What is the relationship between power of magnification and field diameter?

Measuring the Field Diameter of a Compound Microscope.

A compound microscope's field diameter at 100X magnification is too small to be measured accurately with a millimeter scale ruler, even if it were transparent. However, using the mechanical stage of your compound microscope, you can measure, to the nearest tenth of a millimeter, the field diameter. The mechanical stage on your microscope has two scales--one for the x axis and one for the y axis. The smallest division of these scales is 1.0 millimeter (mm). A small vernier with 10 divisions is mounted parallel to each scale. The vernier is used for measuring a fractional part of a division on the scale, and enables you to measure to the nearest tenth of a mm (see Figure 2.4).

X-axis ruler
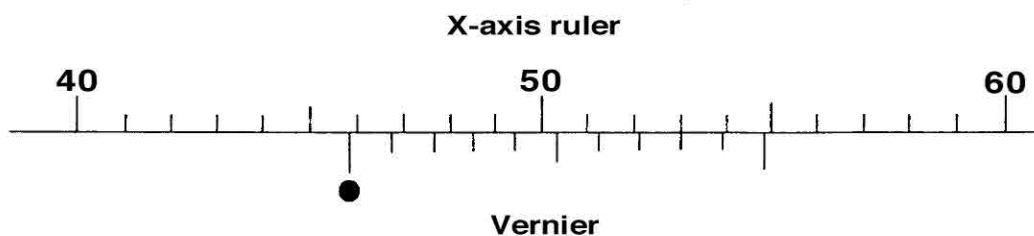
Vernier

Figure 2.4. Portion of the x-axis scale and its vernier on a mechanical stage.

Procedure

1. Using 100X magnification (10X objective) for viewing, focus on the first letter "I" of the **Cornell** slide used previously. Move the slide until the left edge of the letter is tangent with the farthest left border of the field of view (refer to Figure 2.5A). This edge of the letter is your visual reference mark and its position must be recorded with respect to the x-axis ruler of the mechanical stage.

2. Observe on the mechanical stage the region of the x-axis scale adjacent to its vernier. Note the position of the vernier's oval mark on the x-axis scale. Is it directly in line with a mark on the scale, or does it lie between two marks? If the vernier's oval mark is directly in line with a mark on the x-axis scale, the number of that mark is your initial reading. However, if the vernier's oval mark lies between two marks on the x-axis scale, you must determine how many tenths of a mm it is from the nearest mark to the left on the x-axis scale.

For example, in Figure 2.4 the vernier's oval mark lies between the 45- and 46 marks of the x-axis scale; therefore, the reading from the scale is greater than 45, but less than 46. The position of the vernier's oval mark, in tenths from the 45 mark, is determined by finding a mark on the vernier which is in line with a mark on the x-axis scale. The eighth mark to the right of the oval mark on the vernier is in line with a mark on the x-axis scale, hence the initial reading you would record is 45.8.

Aligning the left edge of the letter "I" with the left edge of the field of view and following the procedure described above, record your initial reading on the x-axis scale.

initial reading _____

3. Using the mechanical stage, move the slide along the x-axis until the left edge of the same letter "I" is tangent to the farthest right border of the viewing field (see Figure 2.5B). By this procedure, you have moved your visual reference mark across the diameter of the field of view. Following the procedure described in step 2 above, record your final reading on the x-axis scale.

final reading _____

? The field diameter is the difference between the two readings. What is the diameter (in mm) of the 100X magnification field of view of your microscope?

field diameter (in mm) _____

4. Center the letter "I" in the viewing field and rotate the 40X objective into position above the stage opening. Focus the image.

5. Using the procedures described above, measure the field diameter at 400X magnification:

initial reading _____

final reading _____

field diameter (in mm) _____

Note: The numbers on the x-axis and y-axis scales have no real value; they only denote a place on the axis. The numerical <u>difference</u> between your initial and final readings has value and the units are always <u>mm</u>, regardless of your working magnification. Thus, if you were to measure the width of the letter "o" in Cornell, you would arrive at the same measurement whether at 40X, 100X or 400X.

? Instead of actually measuring the field diameter at 400X, how could you estimate it, if you know the field diameter at 100X?

? What is your estimate of the field diameter at 400X? _____

? What is your estimate of the field diameter at 40X? _____

6. Measure the field diameter at 40X and compare your measured value with your estimated value.

initial reading _____

final reading _____

field diameter (in mm) _____

7.   For most microscopic measurements we need a much smaller unit than the millimeter. The micrometer (μm), which is one-thousandth of a mm (0.001 mm), is used.

?   What is the field diameter at 400X magnification in <u>micrometers</u>? _____
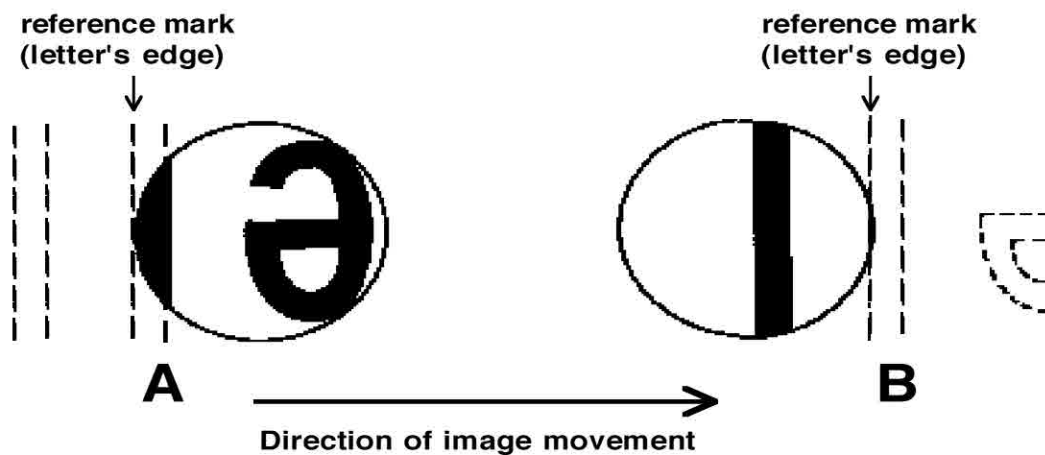
Figure 2.5. Diagrams of the inverted image of Cornell showing initial (A) and final (B) positions of the letter "I" in the field of view in steps 1 and 3, respectively, of the procedure to measure the compound microscope's field diameter. Dashed lines show portions of letters outside the field of view.

<u>Measuring the Size of an Object with the Compound Microscope</u>

1.   Using low magnification (10X objective), center and focus the image of the letter "o" in the field of view.

2.   Measure the width and length of this letter using procedures similar to those described in the previous exercise. To measure the width of the letter, move the slide until the <u>left</u> edge of the letter is tangent to the farthest left border of the field of view. Record your initial reading below. Now move the slide along the x-axis until the <u>right</u> edge of the letter "o" is tangent to the farthest left border of the viewing field. Record your final reading below and determine the width of the letter. To measure the length of the letter move the slide until the <u>uppermost</u> edge of the letter is tangent to the uppermost border of the field of view. Using the y-axis scale record your initial reading below. Now move the slide along the y-axis until the <u>lowermost</u> edge of the letter "o" is tangent to the uppermost border of the viewing field. Record your final reading below and determine the length of the letter.

      <u>Width of Letter "o"</u>   <u>Length of Letter "o"</u>

Initial reading on x-axis scale: _____   **Initial reading on y-axis scale**:   _____

Final reading on x-axis scale: _____   **Final reading on y-axis scale**:   _____

Width of letter "o":   _____   **Length of letter "o"**:   _____

Objects with dimensions smaller than 0.1 mm cannot be measured using the rulers of the mechanical stage. However, an approximate estimate of the size of an object can be made by comparing its dimensions to the diameter of the viewing field. For example, if an object's length is about one-eighth the diameter of the high magnification field, and you have measured that diameter as 400 μm, then the estimated length of the object is about 50 μm.

MICROSCOPIC STUDY OF PLANT CELLS AND TISSUES

Live, Unstained Cells in an *Elodea* Leaf

Elodea is a pond plant which lives completely submerged in water. Its small leaves consist of only two layers of photosynthetic cells, except in the midrib.

❑   Use forceps to remove a healthy leaf from a sprig of *Elodea*. Place the leaf on a clean microscope slide and put 2 or 3 drops of tap water on it. With one hand place the edge of a clean coverslip on the slide near the leaf, and with a finger of the other hand, hold the coverslip edge on the slide. Then gently lower the coverslip onto the leaf. This procedure generally eliminates the trapping of air bubbles in the wet mount preparation.

❑   Place your leaf preparation on the stage of your compound microscope and focus on it with the 4X objective.

You should be able to see distinct cells arranged in rows. Most of the cells are more or less rectangular and contain small green specks.

❑   Scan the width of the leaf and find the narrow, grayish midrib which runs the entire length of the leaf.

❑   Select a portion of the leaf away from the midrib and focus on it with the 40X objective.

❑   Use the fine focus knob to carefully focus up and down through the leaf.

As you do this procedure, you should be able to see two layers of cells. If you cannot, try adjusting the light intensity. The cells in one layer may be more distinct than those in the other layer.

?   Are the cells in the two layers approximately the same size?

?   How could you determine which of the cell layers is the upper one in your preparation? Hint: Carefully rotate the coarse focus knob to determine which way it must be turned to lower the stage; the fine focus knob works the same way.

❑   Carefully focus with the 40X objective on the layer of cells which are more distinct.

You should be able to see a cell wall around each cell and many green chloroplasts within each cell.

?   Do you see other cell structures you can identify?

❑   Be sure that any other structures you see are <u>in</u> a cell and not above or below the cell.

Although we know that these living cells possess many internal structures, most of those structures do not possess light absorbing substances, and consequently are virtually transparent in light microscopy. The photosynthetic pigments in the chloroplasts make them visible.

❑   Attempt to find cells in which the chloroplasts are in motion. A good region in which to look is the midrib and its adjacent cells.

?   Is the chloroplasts' motion random or in a distinct direction?

Chloroplasts and many other cellular inclusions are transported within the cell by special proteins called motor molecules which move along **microtubules.** The movement of cellular organelles and inclusions is called cyclosis. (See p. 112-114 Campbell and Reece, 2008 for additional information.)

❑   Notice in some cells that the chloroplasts move around just inside the perimeter of the cell.

The reason for this confined motion is that a **vacuole** in the middle of each cell occupies most of the cell's volume. In most plant cells the cytoplasm consists of 1) a vacuole containing a solution which is surrounded by a membrane called a **tonoplast** and 2) the **cytosol** which includes all the other internal cellular structures, except the nucleus. The vacuole's tonoplast restricts the cytosol to a thin layer immediately inside the plasma membrane. In some cases the chloroplasts may appear to move across the middle of a cell; actually they are moving in a thin layer of cytosol adjacent to the upper or lower surface of the cell.

❑   Select a part of the leaf between the midrib and the edge, and focus with the 40X objective on a cell layer in which you can see distinct cells all the way across the field of view.

?   The cells you are observing are too small to be measured directly with the mechanical stage scales. How could you determine the average width of the cells you are viewing by using the 400X magnification field diameter you recorded previously?

❑   Use your proposed procedure to calculate the average width of the cells in micrometers (μm). Do your calculations in the space below.

Preserved and Stained Cells in Thin Sections

The thin sections of plant roots which you will observe in these exercises were obtained by the procedures described on p. 16.

When biological materials are sliced thinly enough to be useful for high magnification light microscopy most of them are relatively transparent and lack contrast. Various dyes (or stains) are used to increase contrast in biological specimens. Staining involves the binding of dye molecules to certain molecules in the biological material. Histologists and cytologists have developed numerous techniques which enable them to stain specifically certain structures and substances in tissues and cells. The sections of plant roots that you will observe show the results of using four different staining procedures on the same specimen. Table 2.3 gives information on each of the stains used.

Table 2.3. Four histological stains, their colors and cellular components stained by each.

Stain	Color	Cellular component(s) stained
Fast green FCF	Green	Cellulose* in cell walls
		Cytoplasm
Safranin-O	Red	Lignin* in cell walls
		Nuclear material (chromatin, chromosomes and RNA)
Crystal violet	Violet	Nuclear material
		Starch in amyloplasts
Orange G	Yellow to Orange	Cytoplasm
		Its main function is to differentiate the staining intensities between safranin-O and crystal violet in substances stained by both dyes.

* The walls of all plant cells contain cellulose. Some types of plant cells produce thick secondary walls which are impregnated with a complex polymer called lignin.

❑ Obtain the prepared microscope slides of *Ranunculus acris*: (buttercup) mature root c.s.

While you study these specimens, your lab instructor will project photomicrographs of similar specimens and assist you in identifying certain structures.

❑ Place the 4X objective in position over the stage opening and replace the slide with the one labeled *Ranunculus acris*: mature root. Position a section for viewing and use the 10X objective to observe it.

This cross section of a buttercup root was cut 1 cm or more from the root tip. The thickness of the section is approximately the width of a cell on the surface. Unlike the unspecialized cells in the root section previously observed, most of the cells in this section are specialized to perform various functions in the root.

❑ Observe the central circle of cells surrounded by a ring of cells with thick, red walls.

Most of the cells within the ring are components of tubes which are used to transport substances throughout the length of the root.

❑ Observe the cells containing violet grains.

The violet structures are **amyloplasts**; each is a starch grain surrounded by a membrane. A primary function of these cells located between the central circle and the outer surface of the section is nutrient storage; starch is the major type of stored nutrient.

❑ Observe a projected scanning electron micrograph of a root cross section. Compare your view of a root cross section using light microscopy at 100X magnification to the view obtained using SEM. Notice that the section used for SEM was considerably thicker than the one on your slide.

❑ Observe the central circle of your root cross section at 400X magnification. Note the heavy red walls of cells in the center of the circle. Keep this slide on your microscope for a subsequent exercise.

❑ Observe a projected scanning electron micrograph of similar cell walls obtained at higher magnification and a different viewing angle. Notice that the heavy secondary wall of each cell is a spiral band which winds around the cell.

OTHER TECHNIQUES USED IN LIGHT MICROSCOPY

A Comparison of Bright Field and Dark Field Microscopy (A Demonstration)

The type of light microscopy you will use routinely with the compound microscope is bright field microscopy. In this technique light passes through the substage condenser and microscope slide directly into the objective lens. Any portion of the field of view which does not absorb light or alter the direction of light rays appears bright when the image is viewed. An object in the field of view exhibits contrast by absorbing light in varying degrees and by changing the direction of light rays by reflection, refraction and diffraction (the bending of light around the edge of an object). As mentioned before, some thin biological specimens are so transparent that they exhibit little contrast in the bright field, and require some type of staining to increase their contrast. Most staining procedures kill biological material. **Dark field microscopy** (also called dark ground microscopy) can provide an image with a high degree of contrast

from either living or dead material which is unstained. The optical system is arranged to produce an image with reversed contrast; the image appears bright against a background which is completely dark. In dark field microscopy all of the light that would normally pass through the central portion of the condenser lens directly into the objective is excluded by an opaque disc centered immediately below the condenser lens (see Figure 2.6). The only light passing through the condenser lens enters it near the edge. The light rays are refracted in the lens so that they converge on the region occupied by the object to be viewed. If the direction of the light rays is not altered as they pass through the object, the rays pass outside the acceptance angle of the objective. The only light rays which enter the objective are those that have been refracted of diffracted by components and features of the object. Consequently, the image formed by the objective is bright and shows only those parts of the object that refract and diffract light.

❑   Compare the images of *Ranunculus* (buttercup) root cross sections obtained at 100X magnification using bright field illumination in your microscope and dark field illumination in the demonstration microscopes. Please do not change the objective lens on either microscope.

Refracted and diffracted light from object entering objective

Hollow cone of direct light passing outside acceptance angle of objective

Slide on stage

Central opaque disc in focal plane of condenser
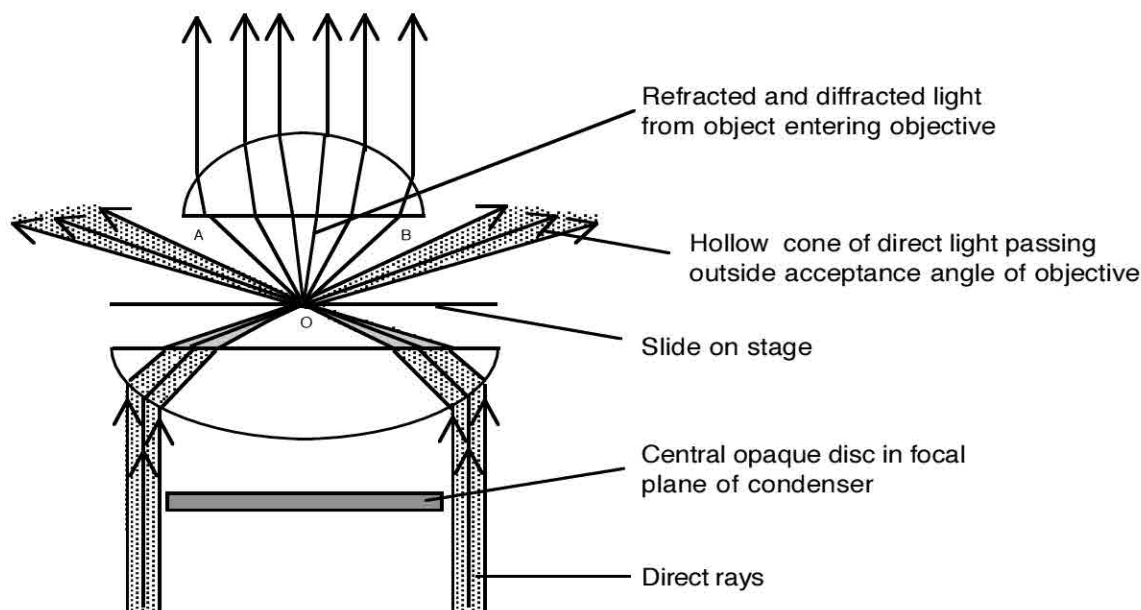
Direct rays

Figure 2.6. Diagram showing a two dimensional view through the middle of the condenser and objective lenses, and the paths of light rays that produce dark field illumination. O is the location of the object. Regions through which direct light passes are shaded. Note that no direct light is within the acceptance angle AOB of the objective. (Adapted from Bradbury, 1976.)

Observation of Plant Structures Using Polarization Microscopy

❑   Obtain two small pieces of polarizing filter mounted on an acetate sheet. Place one on top of the other with their notches together, and look through them at a light source such as the room lights or a window.

?   Is the light's intensity reduced by the filters?

❑   Beginning with the two filters together and their notches in line, observe the light passing through the filters and slowly rotate the filter closer to you 360°. Notice the change in the intensity of transmitted light and how it varies with the orientation of each of the two filters.

? How are the filters arranged to give maximum intensity of transmitted light?

? What arrangement of filters gives the minimum intensity of transmitted light?

Each filter has a polarizing axis that determines the direction of vibration of the light waves passing through it. When the axes of the adjacent filters are parallel, the intensity of light transmitted by them is greatest. When the axis of one filter is perpendicular to that of the other (condition called crossed axes), little or no light is transmitted through the filter positioned farther from the source. In a situation where initially unpolarized light is observed through two polarizing filters, the filter closer to the light source is called the **polarizer** because it polarizes the light waves (i.e., it restricts their vibration to only one direction). The filter farther from the light source is called the **analyzer** because it can be used to determine the vibrational direction of polarized light coming from the **polarizer**. See Figure 2.7.
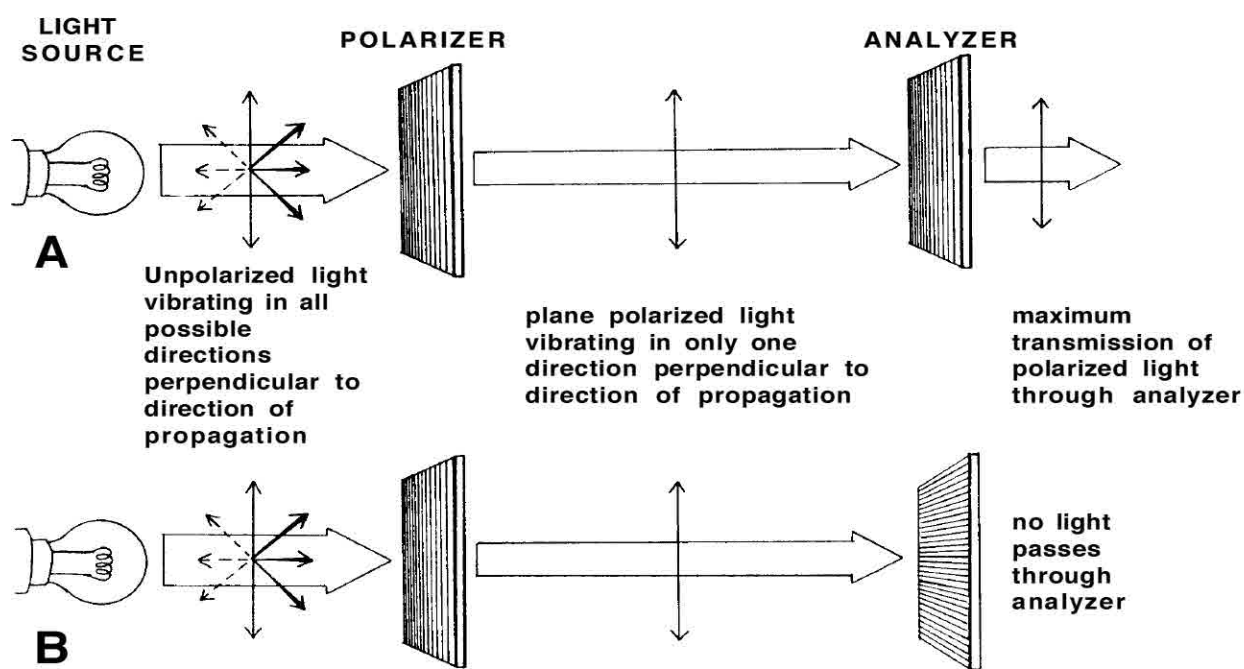
Figure 2.7. The effects of polarizer and analyzer filters on initially unpolarized light. Arrows show the direction of the light wavetrains. Broken lines on arrows mean the direction of the arrows is behind the plane of the page.

    A. **The effect of parallel polarizer and analyzer polarization axes.**

    B. **The effect of perpendicular (crossed) polarizer and analyzer axes. The analyzer will not transmit light whose vibrational plane is perpendicular to the polarization axis of the filter.**

❑ Using 400X magnification, focus on the center (vascular cylinder) of the root section on your microscope. Place one of the filters over the microscope's lamp housing to polarize the light directed toward the root section. Use another filter as an analyzer by holding it on top of the ocular and viewing through it. Start viewing with the filters' axes parallel (determined by the orientation of their notches); then rotate the analyzer until its axis is perpendicular to that of the polarizer.

? Using crossed axes do you see any bright objects in the relatively dark field? Identify these objects.

?   What appearance do these same objects have when viewed with parallel axes?

Discussion of Observations.

In the root cross section you observed with crossed axes of the polarization filters, the bright objects seen are the violet-stained starch granules of amyloplasts and the red-stained heavy secondary cell walls of certain cells. Plant cell walls are composed primarily of cellulose. Both starch and cellulose are composed of long chains of glucose molecules linked together by anhydride bonds. Molecules of starch are deposited in a highly organized manner within starch granules. In the heavy secondary cell walls of plant cells, long cellulose molecules are bundled into microfibrils which, in turn, are laid down parallel to each other in several layers, producing a material with a high degree of directional regularity. Materials with a high degree of atomic or molecular regularity in their structure are called **anisotropic** and can polarize light. When starch granules and heavy cell walls, which are anisotropic, are observed using crossed axes in polarization microscopy, the plane polarized light coming from the polarizer is repolarized by the anisotropic material. Consequently, light passing through the anisotropic material has a polarization plane which is not perpendicular to the polarization axis of the analyzer, and some of the light is transmitted by the analyzer. See Figure 2.8. A more complete discussion of polarization microscopy and these observations is given at the end of this chapter.
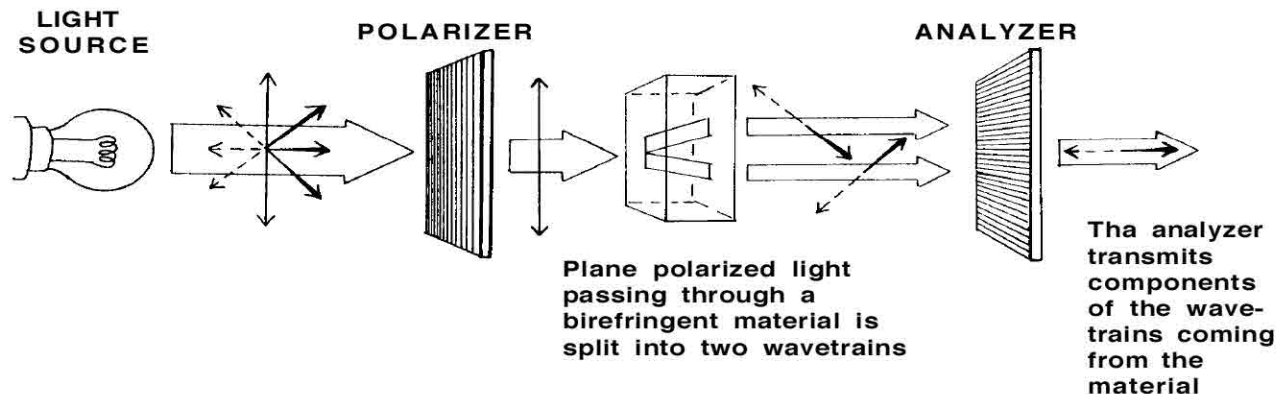
Figure 2.8. The effect of an anisotropic material between a polarizer and an analyzer with crossed axes. The anisotropic material birefracts plane polarized light from the polarizer into two wavetrains whose planes of vibration are perpendicular and different from the vibrational plane of the light transmitted by the polarizer. Since the vibrational planes of the birefracted light are not perpendicular to the analyzer's polarization axis, some of the birefracted light passes through the analyzer.

Observation of Other Biological Structures Using Polarization Microscopy

❑   Observe the following five prepared microscope slide specimens using polarization microscopy: Note: Observe the specimens at 100X magnification. Please do not use the 40X objective.

　　1.　White fibrous tissue, tendon: l. s.

　　2.　Bone dry, ground: c. s.

　　3.　Radiolaria strew

　　4.　Foraminifera strew

　　5.　Pine wood sections

❑   Observe each of the five specimens, first using parallel and then crossed axes of polarizer and analyzer. Compare the objects in the radiolaria strew to objects in the forminifera strew with respect to their interaction with polarized light.

Discussion of Observations

Bone and tendon are two forms of a basic vertebrate tissue type called **connective tissue**. A **tissue** is composed of many cells, which are often similar in structure and function and are held together by intercellular (between cells) material. Connective tissue is characterized by its cells being embedded in an extensive intercellular matrix. In most connective tissues much of the total volume of the tissue is matrix.

Tendons are bands of **dense**, **regular connective tissue** which attach muscles to bones. The fibrous protein, collagen, composes most of the intercellular matrix of dense connective tissue. Collagen molecules are bundled in a parallel arrangement into fibers. In dense, regular connective tissue collagen fibers are parallel and oriented in one direction, so the tissue is anisotropic. Fibroblasts, the cells which synthesize collagen are flattened and packed between adjacent layers of fibers.

The cross section of bone is a thin piece of compact bone from the shaft of a long bone. A cross-sectional view shows several more-or-less circular structures, each composed of concentric rings and a dark central region (see Figure 2.9). In the intact bone each circular structure is a tube called a **Haversian system**. At the center of each Haversian system is a canal which contains blood vessels in the living tissue. Around each canal are a variable number of matrix layers (lamellae) and several cell chambers which contain the bone cells, or **osteocytes**. The cell chambers are connected by thin lines called **canaliculi** (little canals). In the living condition the canaliculi are filled with cytoplasmic extensions of the bone cells. Adjacent cells are in contact with each other through interconnected canaliculi, and cells near the Haversian canal make contact with cells lining the canal through canaliculi. This extensive system of interconnected cells and vascular canals throughout the bone matrix allows substances in solution to move through all living parts of the bone. This morphological feature is what enables bone cells to live in a medium that is solidified by mineral salts.

Haversian canal

osteocyte chambers

canaliculi

Figure 2.9. Cross section of compact bone showing the concentric arrangement of cell chambers and layers of intercellular matrix. The broken line indicates the cross section of one Haversian system.
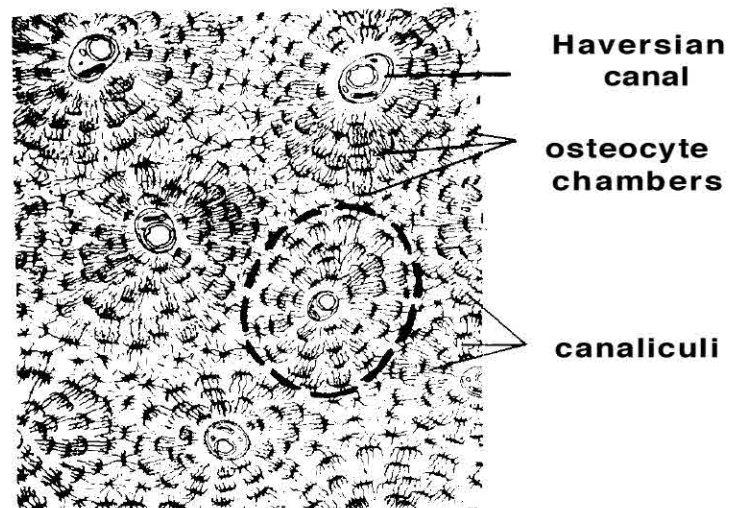
During bone formation osteocytes deposit an intercellular matrix in layers. Each layer consists of parallel collagen fibers oriented in the same direction. Subsequently this matrix is mineralized by the formation of calcium phosphate crystals in it. When you observe bone using polarization microscopy, you can see, in the intercellular matrix, anisotropism from the orderly arrangement of collagen fibers and the regularity of the calcium phosphate crystalline structure.

Radiolarians and foraminiferans are protozoans (unicellular protists). The objects you see on the microscope slides are elaborate skeletons produced by these unicellular organisms. The major component of radiolarian skeletons is silica (silicon dioxide) whose crystalline structure does not have sufficient regularity to make the skeletons anisotropic. Foraminiferan skeletons are composed mainly of calcium carbonate and are anisotropic because of the regularity of the atomic arrangement in the crystalline structure. Complete radiolarian skeletons generally can be distinguished from complete

foraminiferan skeletons by their differences in overall form; however, skeleton fragments cannot be easily identified by their morphology. Polarization microscopy is used by geologists and paleobiologists to distinguish radiolarian from foraminiferan skeleton fragments, in dating sediments.

Note: The following discussion of polarization microscopy is not a required learning objective for this laboratory study. It is provided to supplement your understanding of the principles of polarization microscopy.

Discussion of Polarization Microscopy and Observations Made Using It.

The following is a brief and rather simplified explanation of polarized light and its use in this study. More complete and detailed information can be found in references cited at the end of the chapter or in other physics and microscopy texts.

Light is a transverse wave, like that of a vibrating cord, in which the direction of vibration is perpendicular to the direction of propagation of the wave. Unpolarized light consists of numerous wavetrains vibrating in all possible directions that are perpendicular to the direction of motion; each wavetrain is confined to one plane determined by the direction of vibration. In plane polarized light all the wavetrains vibrate in the same direction (in only one plane). Light-polarizing materials restrict the light wavetrains passing through them to only one or two directions of vibration.

A polaroid sheet contains molecular polymers in a linear parallel arrangement which creates a polarization axis in the sheet. A polaroid filter polarizes light by:

(1)   transmitting those wavetrains whose vibrational direction is parallel to the filter's axis. Due to a slight absorption of the vibrations by the filter, the intensity of the transmitted wavetrains is somewhat lower than that of those entering the filter.

(2)   transmitting a component of each wavetrain whose vibrational direction is at some angle between parallel and perpendicular to the filter's axis. The vibrational direction of the transmitted component is parallel to the filter's axis, and its intensity is lower than that of the original wavetrain entering the filter.

(3)   by not transmitting those wavetrains whose direction of vibration is perpendicular to the filter's axis.

When a polarizer-analyzer pair of filters is used, the analyzer receives polarized light from the polarizer. If the analyzer's axis is parallel to the polarizer's axis (the vibrational direction of the polarized wavetrains), the analyzer transmits the light with a slight reduction in intensity (see Figure 2.7A). If the analyzer's axis is at some angle between parallel and perpendicular to the polarizer's axis, the analyzer repolarizes the light by method 2 mentioned previously. The intensity of the repolarized light is lower than that of the original polarized light because a vibrational component is lost in the repolarization. When the analyzer's axis is perpendicular to the polarizer's axis, no light polarized by the polarizer is transmitted by the analyzer (see Figure 2.7B). When you used polarizer–analyzer crossed axes with the microscope you might have noticed that the field was not completely dark. This is probably due to the entrance of some unpolarized light into the system between the polarizer and the microscope's substage condenser.

Materials with an atomic arrangement having bonds that are randomly oriented are called **isotropic**. Such materials generally cannot polarize light. On the other hand, materials with an atomic arrangement and bonds having a definite pattern of orientation can polarize light and are called **anisotropic**. When light, either polarized or unpolarized, enters an anisotropic material, it is converted into two beams with their planes of vibration perpendicular to each other. Under certain conditions, the two beams pass through the anisotropic material at different velocities and thus, the plane polarized light is doubly refracted by the material. This double refraction is called **birefringence**.

As mentioned previously, when the axes of the analyzer and polarizer are perpendicular to each other (crossed axes), no polarized light coming from the polarizer is transmitted by the analyzer. If an isotropic object is placed on a microscope stage between a polarizer and an analyzer <u>with crossed axes</u>, the field of view seen through the analyzer is dark. However, if an **anisotropic** object is placed between a polarizer and an analyzer <u>with crossed axes</u>, the anisotropic material splits the plane polarized light coming from the polarizer into two beams with planes of vibration perpendicular to each other and with different rates of movement through the material (see Figure 2.8). Usually neither of the beams leaving the anisotropic material has the same plane of vibration as that of the polarized light coming from the polarizer. Since neither of the beams leaving the anisotropic (birefringent) material has a vibrational plane perpendicular to the polarization axis of the analyzer, components of both beams can pass through the analyzer and the birefringent material appears bright in a dark field (see Figure 2.8).

The two types of birefringence commonly seen in biological materials are crystalline birefringence and form birefringence. **Crystalline birefringence** occurs in an anisotropic material whose arrangement of atoms is regular with direction in the crystalline structure. Various calcareous structures (composed of calcium carbonate) produced by organisms exhibit crystalline birefringence when exposed to plane polarized light. **Form birefringence** is due to a regular arrangement of submicroscopic rods or plates surrounded by a medium with a different refractive index.

In the root section, you observed form birefringence in the starch granules of amyloplasts and in the heavy secondary cell walls of certain cells. Plant cell walls are composed primarily of cellulose. Both starch and cellulose are composed of long chains of glucose molecules linked together by anhydride bonds. Molecules of starch are deposited in a highly ordered manner within the starch granules. In the plant cell wall, cellulose molecules are bundled together by cross linking between parallel chains into microfibrils (see p. 73, Figure 5.8 in Campbell and Reece, 2008). In the thin primary cell walls, which all plant cells possess, the cellulose microfibrils are laid down in a somewhat random manner so the directional regularity of the microfibrils is not adequate to produce observable form birefringence in our simple polarization microscope. However, in the heavy secondary walls of some of the cells, the microfibrils are laid down parallel to each other in several layers, producing a material with a high degree of directional regularity. Thus, secondary cell walls are highly anisotropic and their birefringence is readily seen.

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MICROSCOPY REFERENCE SHEET

Procedures for Using the Compound Microscope

1. Move the substage condenser up as high as you can.

2. Clean the ocular, objectives and substage condenser with **lens paper** (nothing else).

3. Lower the stage, if necessary, and mount the slide with the object to be viewed.

4. Turn on the microscope lamp and open the iris diaphragm fully.

5. Use the **4X objective** to find and focus on the object. Position the object so the part you wish to observe is centered in the visual field.

6. Rotate the appropriate objective into using position and adjust the light intensity to observe the object. Use the fine focus knob to focus the image.

7. Remove the ocular lens from the body tube. Look into the body tube and close the iris diaphragm until the diameter of the illuminated circle is 3/4 of its initial diameter.

Steps 3 through 7 should be performed every time a new specimen is used.

8. **Prepare the microscope properly before putting it away.** Lower the stage 1/2 inch or more below the objective. Remove the specimen from the stage. Dry the stage if it is wet. Rotate to the lowest objective, or an empty objective. Place a dust cover over the microscope and return the instrument to its proper storage space.

Useful Information For Using the Compound Microscope

Using 40X magnification (4X objective)

Working distance (between objective and object) = _____ (from p. 21)

Field diameter = _____ (from p. 26)

Using 100X magnification (10X objective)

Working distance = _____ (from p. 22)

Field diameter = _____ (from p. 25)

Using 400X magnification (40X objective)

Working distance = _____ (from p. 22)

Field diameter = _____ (from p. 25)

POST-LAB WEB ASSIGNMENT

To test your understanding of certain concepts and procedures in this laboratory, complete the associated web activities located in the lab section of the course web site.

URL = http://biog-101-104.bio.cornell.edu/BioG101_104/tutorials/microscopy.html

You will encounter questions similar to these on major quizzes and the practical examination.

** NOTES ON MICROSCOPY**

MICROSCOPY WORKSHEET

NAME: _____   **TA:**_____

1.   Assume that the 10X and 40X objectives on your compound microscope have the same diameter.

    A.   Which objective has the greater acceptance angle when it forms an image? (Refer to figure 2.2.) Explain your answer.

                    _____

    B.   What effect would a greater acceptance angle have on resolving power? Explain your answer.

2.   Give the numerical aperture (N.A.) of the 10X and 40X objectives. (Obtain data from p. 22.)

    N.A. of 10X objective = _____   N.A. of 40X objective = _____

3.   If the entire light spectrum (wavelengths from 400 nm to 700 nm) is used, which objective (10X or 40X) has the smaller (i.e. better) resolving power? (See equation 1 on p. 14.)

                    _____

    Calculate the smallest resolving power of that objective. (Show calculations.)

        Resolving power =

4.   A.   What is the relationship between power of magnification and diameter of the viewing field of a microscope? (See page 24.)

    B.   What is the viewing field diameter when you use 10X magnification on the dissecting microscope? (Obtain datum from p. 24.)

                    _____

    C.   Use the distance given in part B to calculate the field diameter observed at 50X magnification (show calculations).

        Field diameter at 50X magnification =

5.   A.   What is the diameter (in mm) of the 100X magnification field of view?_____
           (Obtain datum from p. 25.)

      B.   What is the measured diameter (in mm) of the 400X magnification field of view?_____
           (Obtain datum from p. 25.)

      C.   Instead of actually measuring the diameter of the 400X magnification field, how could you
           determine it, if you know the diameter of the 100X magnification field?

      D.   What is the 400X magnification field diameter obtained from your alternate procedure?_____

      E.   Is your answer to part D the same as the measured diameter (answer to part B)? If there is a
           difference, explain it.

6.   Assume you are observing two crossed threads, a red one and a blue one, with the compound
      microscope. The image of the blue thread is in focus; the image of the red one is not. As you raise
      the stage toward the objective with the fine focus knob, the image of the blue thread goes out of
      focus and the image of the red one comes into focus. Which thread is the upper one? Briefly
      explain your answer.

7.   A.   The *Elodea* leaf cells you observed were too small to be measured directly with the
           mechanical stage rulers. How could you determine the <u>average</u> <u>width</u> of the cells you viewed
           by using the 400X magnification field diameter you calculated in question 5? (Refer to p. 28.)

      B.   Use your proposed procedure to calculate the average width of the cells in <u>micrometers</u> (*µ*m).
           Show your calculations.

CHAPTER 3 - ENZYMES: TESTING A MODEL

LABORATORY SYNOPSIS

In the first chapter you read about scientific methodology. The essence of scientific work (and the foundation of all research) is the testing of an hypothesis. In this laboratory you will test an important prediction of a model describing how enzymes interact with substrate molecules and speed up biological reactions. You will use a spectrophotometer to measure the color change of reactions catalyzed by the enzyme alkaline phosphatase at different substrate concentrations. You will convert color change measurements to product concentration by using a standard curve and will then compare your results with those predicted by the hypothesis.

LEARNING OBJECTIVES

Conceptual

1. Draw a curve to show changes in free energy of reactant molecules as they become product molecules in an energy-yielding reaction.

2. Define activation energy, transition state, and net reaction energy change, and explain how these terms relate to the curve described in Objective 1.

3. Show how enzyme catalysis changes the curve described in Objective 1.

4. Write the general reaction describing how enzymes interact with substrates and accelerate reactions.

5. Describe what a standard curve is, how it is obtained, and how you will use it to estimate the amount of product formed in your reaction systems.

6. Define the term "buffer".

7. Describe the effect of pH on enzymes and how pH is used in this study to stop reactions and create a blank for the spectrophotometer.

8. Draw the curve showing the relationship between substrate concentration and reaction velocity. Describe the three portions of the curve where free enzyme exists, where enzyme limits the reaction, and where all enzyme is saturated with substrate.

9. Explain the law of mass action, its predictions for the relationship between reactant concentration and reaction velocity, and why enzyme catalyzed reactions deviate from those predictions.

10. Define maximal velocity (V_m) and the Michaelis-Menten constant (K_m) and estimate these values from a substrate concentration-velocity curve.

11. Identify substrate concentration-velocity curves and K_m constants for enzymes with low, medium, and high efficiency as catalysts.

12. Explain the effect of enzyme concentration on the V_m and K_m of an enzyme.

13. Explain the effect of competitive and noncompetitive inhibitors on the V_m and K_m of an enzyme.

Methodological

1.   Properly set up and use a spectrophotometer to measure absorbance of test solutions relative to a blank.

2.   Use the spectrophotometer, a set of solutions of known concentration for a substance, and graph paper to produce a standard curve for the substance.
3.   Examine an absorption spectrum or a transmission spectrum of a substance in solution and select the wavelength that optimizes the sensitivity of the spectrophotometer for detecting that substance.

4.   Use the standard curve to estimate the concentration of a test solution based on the solution's absorbance.

5.   Gain experience with the use of digital micropipettors.

READING ASSIGNMENT

       In addition to reading this chapter before laboratory, you should also read Appendix 2 (pp. A3-A8) on the theory and operation of the spectrophotometer. It may help to review Campbell and Reece (2008) section on enzymes, pp. 151-159.

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

1.   In this study you are testing the model explaining how enzymes work as catalysts by determining if one of its predictions is true. What is the prediction being investigated?

2.   Why is 410 nanometers the best wavelength of light to use in estimating the concentration of *p*-nitrophenol, the product of the reaction you will be studying in this laboratory?

3.   In using the standard curve in this study, what quantity do you know and what quantity are you trying to estimate?

4.   Why are you using absorbance to estimate product concentration instead of percent transmittance (%T)?

5.   Why do you add 0.2 N NaOH to the blank solution <u>before</u> you add enzyme?

6.   Why do you set the spectrophotometer to 100%T with the blank solution in the sample holder before measuring the absorbance of the test solution?

7.   In using the spectrophotometer in this study, why must each test solution have its own blank solution?

8.   If a reaction system is at maximal velocity, what is the relationship between enzyme, substrate, and enzyme-substrate complex?

Jon C. Glase

Revised June 2010
Mark A. Sarvary

INTRODUCTION

The chemical reactions occurring within cells are exactly like those taking place in nonbiological situations, with one important exception: reactions within cells are accelerated by unique biological catalysts called **enzymes**. In this laboratory topic, you will consider a model that explains how enzymes function as catalysts and will collect some data to test an important prediction of this model. A **model** is simply a research hypothesis expressed in the form of an equation or formula, or with reference to an analogous physical process.

Chemical Reaction

Before discussing how enzymes function, we will briefly review general information about chemical reactions by considering the following reversible reaction:

<u>Reactants</u>   <u>Products</u>
A + B   ↔   C + D

(a) without enzyme   **(b) with enzyme**
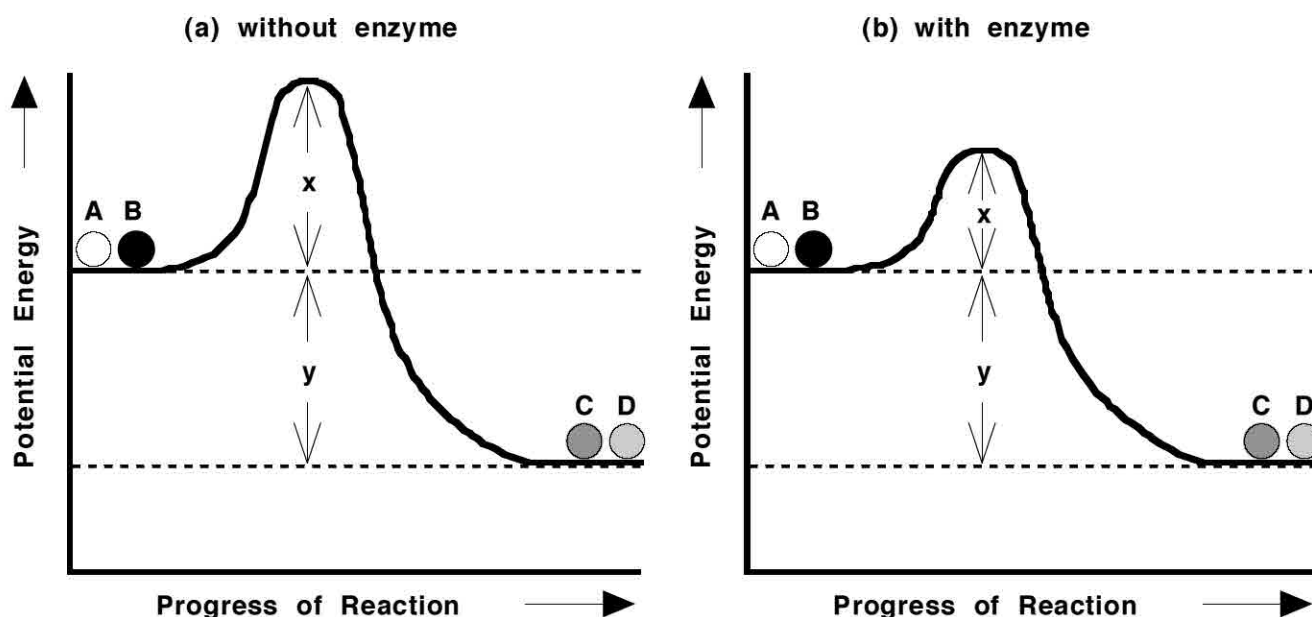
Figure 3.1. Change in the free energy of reactant molecules A and B as they enter a transition state and become product molecules C and D, both without (a) and with (b) enzyme catalysis (x = activation energy; y = net free energy change or ΔG).

If we define **free energy (G)** as energy available to do work, then, as shown in Figure 3.1, the free energy of an A molecule and a B molecule is greater than that of a C molecule and a D molecule. Therefore, the net reaction will proceed from left to right, and it will be an energy-yielding, or **exergonic**, reaction. However, before A and B can form a chemical bond, they must collide with sufficient force to enter a **transition state**. The energy required to enter the transition state is called the **activation energy**. The activation energy for the **forward reaction** equals **x** in Figure 3.1a; **y** is the **change in free energy (ΔG)** resulting from the reaction. The **back reaction** is an energy-requiring or **endergonic** reaction. The activation energy required for the back reaction is equal to **x + y**.

The rate of the reaction can be increased by adding energy to the system so that more A and B molecules have the activation energy needed to enter the transition state. Outside of cells this can be done in three ways without using a catalyst: the mixture of A and B molecules can be heated, the pressure on the system can be increased, or the concentration of reactants can be increased. Each method increases the collision rate of A and B, thus increasing the number of A and B molecules entering the transition state.

Cells do not use temperature to increase the rates of reactions because high temperatures destroy the large macromolecules of which the cell is composed. Rather, living systems must conduct "cold chemistry". Pressure is also not generally used to accelerate reaction rates within the aqueous systems found in cells. Cells do accumulate reactants as a way of accelerating reactions, but the main method they use is catalysis with enzymes, which carefully position the reactants.

Enzyme-Catalyzed Reactions

Enzymes are large globular proteins ranging in molecular weight from 10,000 to over 500,000 daltons. If we assume that the average molecular weight of amino acids is 120 daltons, enzymes consist of 80 to over 400 amino acids. The folding and refolding of the amino acid chains, held together by hydrogen and disulfide bonds, give the enzyme a three-dimensional globular shape which includes a depression or cleft into which the reactant molecules fit. This area of the enzyme is called its **active site**.

Enzymes function as catalysts by increasing the efficiency with which reactants interact. When the reactants are attached to the enzyme's active site, they are arranged so that their reactive regions are precisely aligned. Alignment of reactants by the enzyme allows the reactants to enter the transition state at a lower energy level. Thus enzymes lower the amount of activation energy needed for the reaction to occur, as shown in Figure 3.1. Notice that the reduction in activation energy by the enzyme accelerates the forward and back reactions equally.

Reactants that are acted on by enzymes are called **substrate** molecules. The temporary union of enzyme and substrate, when the substrate is attached to the enzyme's active site, is called the **enzyme-substrate complex.** Because the shape of the enzyme's active site depends on the sequence of amino acids in the polypeptide chains and the precise folding of chains caused by bonding between chains, enzymes are usually specific for those types of substrates that are able to attach to the active site. Therefore, most enzymes catalyze only a single reaction or group of similar reactions.

We can represent the model for enzyme catalysis as a generalized reaction

$$E + S \leftrightarrow ES \leftrightarrow EP \leftrightarrow E + P$$

where: E = enzyme
             S = substrate(s)
             ES = enzyme-substrate complex
             EP = enzyme-product complex
             P = product(s)

This model suggests that S becomes P by transition through ES and EP and also that E remains unchanged after the reaction is over. The double headed arrows indicate that this is a reversible reaction but that in the absence of product the net reaction will be from left to right. The model also ignores the small amount of P formed by the noncatalyzed conversion of S directly into P because of the very low rate of this reaction.

With a fixed number of enzyme molecules in the system and relatively little substrate (Figure 3.2), adding more substrate (increasing S) will increase reaction velocity (v). Thus, we would initially expect a nearly linear relationship between substrate concentration ([S]) and v (see Figure 3.3, part "a" of the curve). However, at greater [S], fewer free enzyme molecules will be available to combine with the substrate. That is, the lack of available enzyme limits the reaction and yet more substrate must be added

to "find" the remaining free enzyme molecules (Figure 3.2b). We can then predict that the initial near linear relationship between [S] and v should start to level off (Figure 3.3, part b of the curve) in systems with more and more substrate. Finally, the level of S will **saturate** all the enzyme present, that is, all the E will be in the ES or EP complex (Figure 3.2c). When all the enzyme is saturated with substrate, further increasing S will not increase v (Figure 3.3, part c of the curve) and the maximal velocity for the reaction.

This model for enzyme catalysis, if correct, makes one important prediction regarding the relationship between substrate concentration, [S], and the rate of the reaction (V). Because catalysis by the enzyme depends on the formation of an enzyme-substrate complex at the active site of the enzyme, and because the total number of active sites may be limited, it is possible for there to be **saturation**. Saturation occurs when the concentration of substrate is so high that all the active sites of all the available enzyme molecules are fully occupied. Thus, for a given concentration of enzyme that is at saturation, the addition of a still higher substrate concentration will not result in a higher rate of enzyme catalysis.

Saturation is something that is not predicted for uncatalyzed reactions. The relationship between the concentration of reactants and rate of an uncatalyzed reaction is described by the **law of mass action**. As stated earlier, more reactants result in more collisions between reactants thereby increasing the number entering the transition state and being converted to product. Therefore, in a noncatalyzed reaction, increasing the concentration of reactants will always increase v. However, in a reaction with enzyme catalysis, reactants (substrate) must temporarily combine with E to form ES and then EP before conversion to product and this requirement leads to different results than those predicted by the law of mass action. Remember that a reaction can occur in a catalyzed <u>and</u> in an uncatalyzed manner.

(a) (b) (c)

◠ **Enzyme** ○ **Substrate** ◠ **Enzyme-substrate complex**
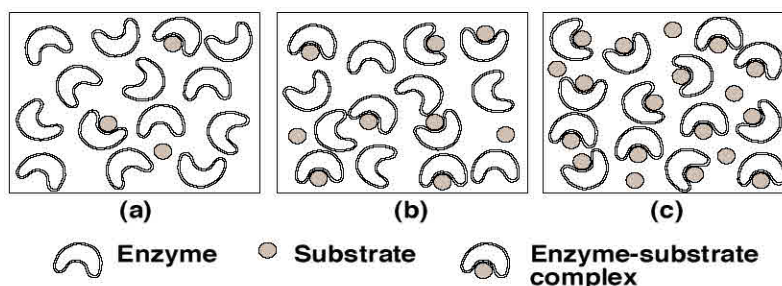
Figure 3.2. The binding between enzyme and substrate in systems with (a) many free enzyme molecules, (b) limited enzyme molecules available for substrate, and (c) all enzyme saturated with substrate.
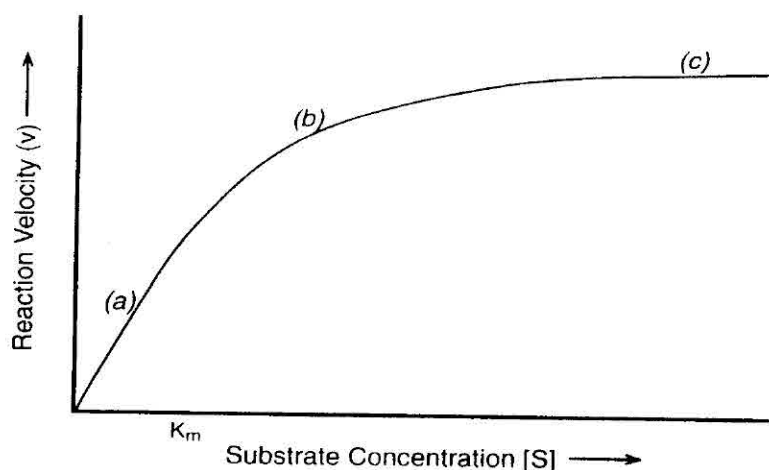
Figure 3.3. The relationship between reaction velocity (v) and substrate concentration [S] when (a) many free enzyme molecules exist, (b) enzyme availability limits the reaction, and (c) all enzyme is in the enzyme-substrate (ES) or enzyme-product (EP) complex.

In today's study you will test this model for enzyme catalysis by measuring reaction rates at various substrate concentrations and comparing the relationship between [S] and v with what the model predicts (as shown in Figure 3.3). To do this, you will set up a number of different reaction systems, each with a different substrate concentration. All the other conditions within the systems, such as pH, temperature, reaction time and enzyme concentration, will be identical; therefore any observed differences can be attributed to the effect of substrate concentration.

Alkaline Phosphatase

Phosphatases are enzymes that form enzyme-substrate complexes with compounds called phosphoric acid esters. All of the reactions catalyzed by phosphatases are hydrolysis reactions in which a water molecule is added to the substrate and a phosphate ion is liberated. You will be working with the enzyme **alkaline phosphatase**, so called because the enzyme functions best at an alkaline pH of 10.5. Another group of phosphatases, the acid phosphatases, function best under acidic conditions (pH < 7).

The specific reaction you will study involves the hydrolysis of a colorless substrate, ***p*-nitrophenyl phosphate**, to a yellow product, ***p*-nitrophenol**, plus phosphate ion. *p*-nitrophenyl phosphate is a "laboratory substrate" not found naturally in cells. It is used in this study because the presence of its colored product provides a convenient, accurate means for measuring the rate of the reaction. In organisms, alkaline phosphatase reacts with naturally-occurring phosphoric acid esters and is involved in the transport of phosphate into and out of cells. Alkaline phosphatase is especially abundant in the cells of tissues and organs concerned with nutrient transport, such as bone, the placenta, the lining of the gut, and the mammary glands. The intestinal mucosa of the chicken was the source of the purified enzyme you will be using in today's laboratory.

p-nitrophenyl phosphate   *p*-nitrophenol   phosphate
            (colorless)   (yellow)   ion
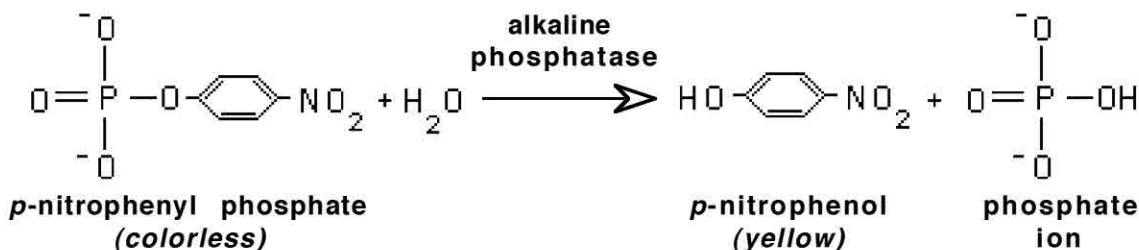
Effect of pH on Enzymes

Enzymes depend on their three-dimensional shape to function properly; any factor that affects the shape of an enzyme will drastically alter its ability to act as a catalyst. The enzyme's shape is maintained primarily by hydrogen bonding between the polypeptide chains; consequently, the pH, or hydrogen ion concentration, of the environment has a direct effect on the enzyme. If the pH is much above or below the optimum range for an enzyme, the enzyme functions much less efficiently and may even be destroyed (**denatured**). A **buffer** is a substance that binds with or releases hydrogen ions to maintain a specified pH despite the addition or removal of hydrogen ions from the system. In this laboratory study we will use a buffer system consisting of the amino acid glycine which maintains a pH of about 10.5, optimum for alkaline phosphatase.

The pH sensitivity of enzymes can be used as a way of stopping reactions. In this study, you will use 3.0 ml of a strong base, 0.2 N NaOH, to stop the reaction. This base removes most of the H^+ from the system, overpowers the buffering capacity of glycine, and denatures all alkaline phosphatase molecules present. NaOH is also used in preparing the blank for the spectrophotometer, as discussed below.

MEASURING VOLUMES

In this study, you will be using **digital micropipettors** to measure and dispense all liquids. You will use a 1–5 ml micropipettor set to 1.0 ml to add buffered substrate to each cuvette. You will start reactions by using a 20–200 μl micropipettor set to 100 μl (0.1 ml) to add enzyme to cuvettes containing the buffered substrate. After a set time interval, you will stop reactions by using a 1–5 ml micropipettor set to 3.0 ml to add 0.2 N NaOH to reaction systems.

❑   Read the section of Appendix 2 that describes the use of digital micropipettors. Your lab instructor will demonstrate proper use of this instrument.

STANDARD CURVE

The color change occurring in the reaction system as colorless substrate is converted to the reaction's colored product, p-nitrophenol, will be measured with an instrument called a spectrophotometer (see Appendix 2, page A3). However, before the color change of a reaction system can be related to a specific product concentration, you must construct a standard curve. A standard curve shows the relationship between the concentration of a substance and some physical characteristic of the substance that varies directly with its concentration. In the standard curve for p-nitrophenol, you will establish the relationship between known concentrations of p-nitrophenol solutions and their absorbance of light at a wavelength of 410 nanometers (nm) (the wavelength best absorbed by p-nitrophenol). You will work with a set of standard curve solutions, including five known concentrations of p-nitrophenol (2.5, 5.0, 10.0, 25.0, 50.0 μM) and a blank.

❑   Using the outline in Appendix 2 on the spectrophotometer (pages A5–A8), set the wavelength-selector knob to 410 nm and determine the % transmittance and absorbance of the five p-nitrophenol concentrations. Record your values in the following table:

p-nitrophenol Concentration	**% Transmittance**	**Absorbance**
2.5 μM		
5.0 μM		
10 μM		
25 μM		
50 μM		

❑   To obtain the standard curve for p-nitrophenol, plot the relationship between absorbance of light at 410 nm and p-nitrophenol concentration. Label the graph axes properly and **provide a complete title for Figure 3.5** (see Appendix 3 on Scientific Writing, page A21-A23). The resulting curve should be linear; if not, see your lab instructor for help.
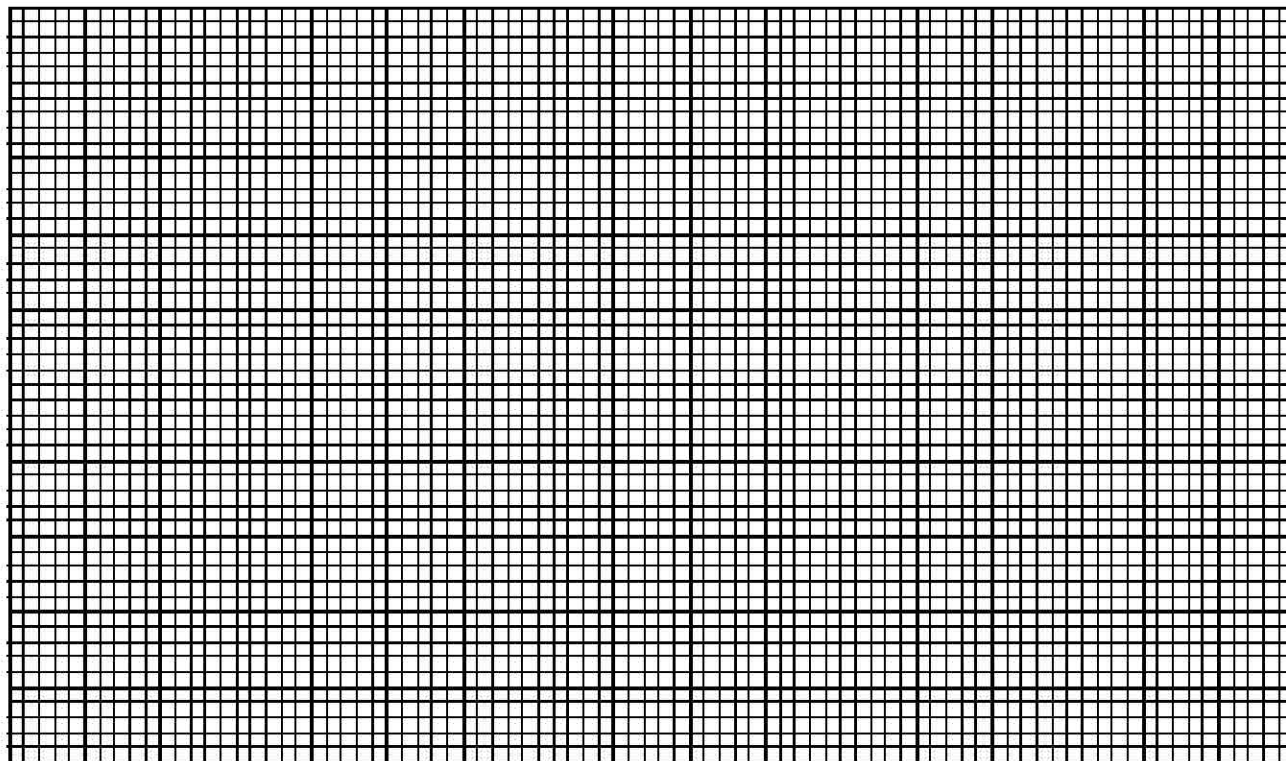
Figure 3.5.

❑   The standard curve in Figure 3.5 can now be used to estimate product concentration when only the absorbance of the solution is known. Later, you will use this curve to estimate the concentration of p-nitrophenol based on the measured absorbance of the reaction systems in this study.

PROCEDURE

In this study you will set up seven pairs of cuvettes containing the reaction systems. Each cuvette pair will contain a different substrate concentration. You will add enzyme to the **test** cuvette of each pair and allow the reaction to proceed for 15 minutes at 37° C (this is the optimum temperature for most enzymes extracted from warm-blooded animals). You will add NaOH to the **blank** cuvette of each pair before you add the enzyme. When enzyme is added, the high pH in the blank cuvette mixture will denature the enzyme and no reaction will occur. Thus, the blank cuvette will contain everything present in the test cuvette, except product. For each pair, the blank cuvette will be used to set up the spectrophotometer so that only light absorption by product is measured in the test cuvette. You will then use your standard curve to estimate product concentration based on absorbance. A flow chart for this study is shown in Figure 3.6. Detailed instructions are given below. You will be working in pairs and each pair will run a set of reactions at a different enzyme concentration (0.5, 1.0, 1.5, or 2.0 mg/100 ml). Each pair should do the following:

❑   **Setting up the Reaction Systems**

   a.   Prepare 14 cuvettes by placing a small piece of masking tape on the upper end of each cuvette. Using a pencil, number the cuvettes.

b. Using a 1–5 ml micropipettor (labeled 1000-5000 μl set to 1.0 ml, add to each pair of cuvettes 1.0 ml of a different substrate concentration, as shown in the flow chart. You can use the same pipette tip for the entire operation, by <u>pipetting from the least to the most concentrated</u> substrate solution. All substrate solutions are buffered at pH 10.5 by a 0.1 M glycine buffer.

c. Using a 1–5 ml micropipettor set to 3.0 ml, add 3.0 ml of 0.2 N NaOH to the even-numbered cuvettes (cuvettes 2, 4, 6, 8, 10, 12 and 14 will be your <u>blanks</u> for use in the spectrophotometer).

d. Place all 14 cuvettes into a 37° C water bath for a minimum of 5 minutes.

❑   **Starting the Reactions**

Perform the following procedure with each of the 7 odd-numbered, <u>test</u> cuvettes (1, 3, 5, 7, 9, 11, 13) one at a time:

a. Remove the cuvette from the water bath.

b. Add the enzyme solution (0.5, 1.0, 1.5, or 2.0 mg/100 ml) to each test cuvette with a 20-200 μl digital micropipettor set to 100 μl (0.1 ml). Cap the cuvette with a piece of precut parafilm and invert to mix. Use the same pipette tip for adding enzyme to all 7 test cuvettes. (NOTE: To be efficient, start a reaction in one test cuvette <u>every</u> <u>minute</u>.)

c. Note the time and return the cuvette to the water bath for <u>exactly 15 minutes</u>.

❑   **Preparing the Blank Cuvettes (can be accomplished while reactions are running)**

First, eject the used pipette tip and insert a new tip onto the micropipettor.

Perform the following procedure with each of the 7 <u>blank</u> cuvettes (2, 4, 6, 8, 10, 12, 14):

a. Remove the cuvette from the water bath.

b. Add the enzyme solution (0.5, 1.0, 1.5, or 2.0 mg/100 ml) to each blank cuvette with a 20-200 μl digital micropipettor set to <u>100</u> μl (0.1 ml). Cap the cuvette with a piece of precut parafilm and invert to mix. Use the same pipette tip for adding enzyme to all 7 blank cuvettes.

c. Return the cuvette to the 37° C water bath. <u>Note</u>: since very little reaction will occur in the blank cuvettes, due to the absence of functional enzyme, you do not need to record time spent in the water bath.

❑   **Stopping the Reactions**

Perform the following procedure with each of the 7 test cuvettes (1, 3, 5, 7, 9, 11, 13):

a. After 15 minutes remove the cuvette from the water bath.

b. Using a 1–5 ml micropipettor set to 3.0 ml, add 3.0 ml of 0.2 N NaOH to saturate the buffer system and stop the reaction. Recap the cuvette with parafilm, and invert several times to mix.

c. Carefully dry the outside of the cuvette, and set it aside for analysis with the spectrophotometer.

d. Remove the blank cuvettes from the water bath, dry the outside of each cuvette, and set them aside for use in the spectrophotometer.

❑   **Determining Absorbance**

You will use the spectrophotometer to compare each test cuvette with its corresponding blank cuvette (see Appendix 2). For <u>each pair</u> of cuvettes, do the following:

a.   Set the spectrophotometer to 0.0% T (with the sample holder empty) at a wavelength setting of 410 nm.

b.   Insert the blank cuvette in the sample holder, and adjust the instrument to read 100% T.

c.   Insert the appropriate test cuvette and read % T to the nearest 1/4% T. Convert % T to absorbance using Table 1 in Appendix 2, page A8.

❑   **Estimating Reaction Velocity**

To estimate the p-nitrophenol concentration in each test cuvette, do the following:

a.   Find the point on the vertical (y) axis of the standard curve (Figure 3.5) that equals the observed absorbance for the test cuvette.

b.   Note where a horizontal line from that point intersects the standard curve line.

c.   A vertical line from this intersection point to the horizontal (x) axis will show you the p-nitrophenol concentration with the solution's absorbance.

d.   Divide product concentration by 15 minutes to express the reaction velocity obtained per minute.

e.   Enter these data on Table 3.1.

❑   **Plotting the Results**

Plot your group's data from Table 3.1 in Figure 3.7. Draw a curve that best fits these data. Label the graph axes properly and **write an appropriate title** for the figure.

Substrate Concentration (M)

| 0.003 | 0.006 | 0.012 | 0.024 | 0.048 | 0.060 | 0.100 |

☐ Add substrate
volume to add = 1.0ml
(use 1–5 ml micro-
pipettor set to 1.0 ml)

1.0 ml 1.0 ml 1.0 ml 1.0 ml 1.0 ml 1.0 ml 1.0 ml

☐ Add NaOH to blanks
volume to add = 3.0ml
(use 1–5 ml micro-
pipettor set to 3.0 ml)

3.0 ml 3.0 ml 3.0 ml 3.0 ml 3.0 ml 3.0 ml 3.0 ml

Setting up the reaction systems

☐ Place in water bath
(37° C), 5 minutes

☐ Remove test cuvette
☐ Add enzyme:
volume to add = 0.1ml
(use a 20-200 µl micro-
pipettor set to 100 µl)

0.1 ml 0.1 ml 0.1 ml 0.1 ml 0.1 ml 0.1 ml 0.1 ml

☐ Return to water bath

Starting reactions

☐ Add enzyme to blanks
volume to add = 0.1ml
(use a 20-200 µl micro-
pipettor set to 100 µl)

0.1 ml 0.1 ml 0.1 ml 0.1 ml 0.1 ml 0.1 ml 0.1 ml

☐ Return to water bath

Preparing the blanks

☐ After 15 minutes,
add NaOH to test
cuvettes
volume to add = 3.0ml
(use 1–5 ml micro-
pipettor set to 3.0 ml)

3.0 ml 3.0 ml 3.0 ml 3.0 ml 3.0 ml 3.0 ml 3.0 ml

Stopping reactions

☐ Compare pairs of
cuvettes in the
spectrophotometer

Determining absorbance

Figure 3.6. Flow chart for the study of the effect of substrate concentration on the velocity of an enzyme catalyzed reaction (see the text for a detailed description of procedures).

Table 3.1. Data from the study of the effect of substrate concentration on reaction velocity for the enzyme alkaline phosphatase at a concentration of _____ **mg/100 ml.**

Cuvette Number	Sub. Conc. (mM)	% Transmittance	Absorbance	Product Conc. (μM)	Velocity (μM/min)
1	3				
2	3	100	–	0.0	0.0
3	6				
4	6	100	–	0.0	0.0
5	12				
6	12	100	–	0.0	0.0
7	24				
8	24	100	–	0.0	0.0
9	48				
10	48	100	–	0.0	0.0
11	60				
12	60	100	–	0.0	0.0
13	100				
14	100	100	–	0.0	0.0
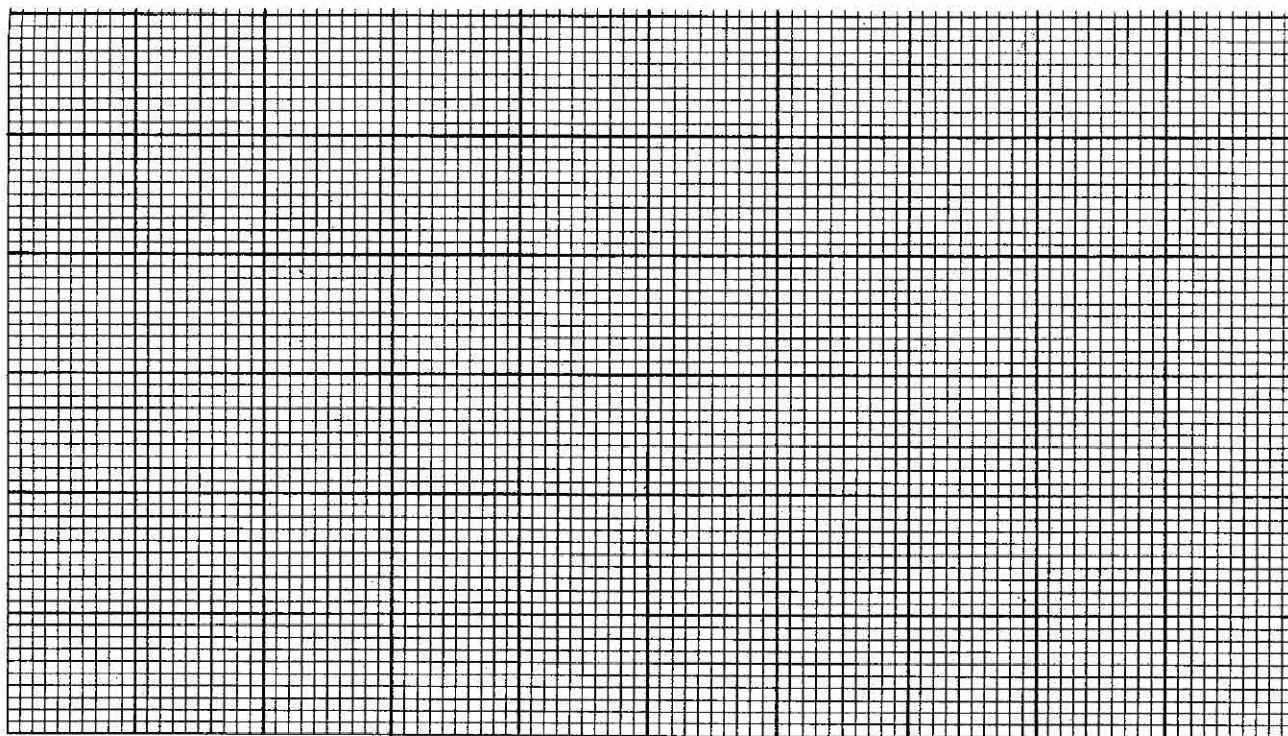
Figure 3.7.

DISCUSSION OF RESULTS

Testing the Model

Examine Figure 3.3 as we review the expected results for this study. At low substrate concentration [S] there will be a linear relationship between [S] and reaction velocity (v); an increase in [S] should produce a proportional increase in v. At low [S] many free, unoccupied enzyme molecules are available, and adding more substrate will allow these enzyme molecules to function as catalysts and speed up the reaction. At higher [S], however, fewer free enzyme molecules remain, and it becomes increasingly difficult for substrate molecules to combine with enzyme. Thus, the relationship between [S] and v becomes less linear as [S] is increased. Eventually all of the enzyme molecules are occupied with substrate or product; that is, as soon as EP → E+P, E immediately recombines with more S to form ES. When all enzyme is **saturated** with substrate or product (ES or EP), the **maximal velocity** (V_m) is reached. Once it is reached, further increasing [S] will have no effect on v via catalyzed reactions because all the enzyme in the system is saturated.

? How does your curve in Figure 3.7 compare with the curve in Figure 3.3 showing the expected relationship between substrate concentration [S] and reaction velocity (v)?

❑ Using the data plotted in Figure 3.7, estimate V_m, expressed in appropriate units. To determine V_m from your data, you need to estimate the reaction velocity reached when the curve becomes horizontal (see Figure 3.8).

$V_m=$
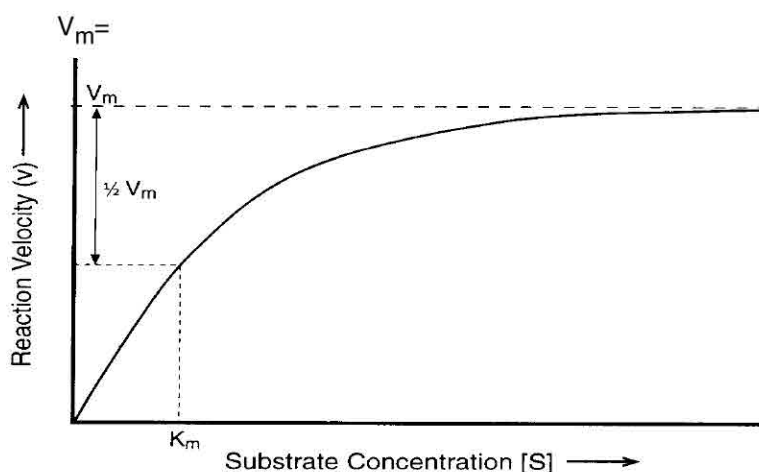
Figure 3.8. Estimation of the maximal velocity (V_m) and Michaelis-Menten constant (K_m) from a substrate concentration-velocity curve.

Another important prediction of the model for enzyme catalysis is that the magnitude of V_m should depend on the number of enzyme molecules present in the system. As enzyme concentration increases, V_m increases and the [S] needed to reach V_m increases. Generally, reaction rates within cells are limited by the number of enzyme molecules present. Thus, cells not only determine the types of reactions occurring by the <u>kinds</u> of enzymes they produce, but they also regulate the speed of these reactions by the <u>number</u> of enzyme molecules produced.

Different enzymes vary in the efficiency with which they catalyze reactions, depending on how quickly the enzyme combines with substrate and releases product. Highly efficient enzymes reach a V_m at lower [S] than less efficient enzymes. Thus, the substrate concentration-velocity curves for highly efficient enzymes are located to the <u>left</u> of curves for enzymes that are less efficient catalysts (see Figure 3.9).

The Michaelis-Menten Constant

A standard method for characterizing the catalytic efficiency of an enzyme is to estimate its **Michaelis-Menten constant**. The Michaelis-Menten constant, or K_m, is the substrate concentration required to obtain one-half the maximal velocity. Figure 3.8 shows how to estimate K_m from graphical data, and Figure 3.9 shows how K_m varies for enzymes with different catalytic efficiencies. Note that the greater the enzyme's effectiveness as a catalyst, the smaller the K_m.
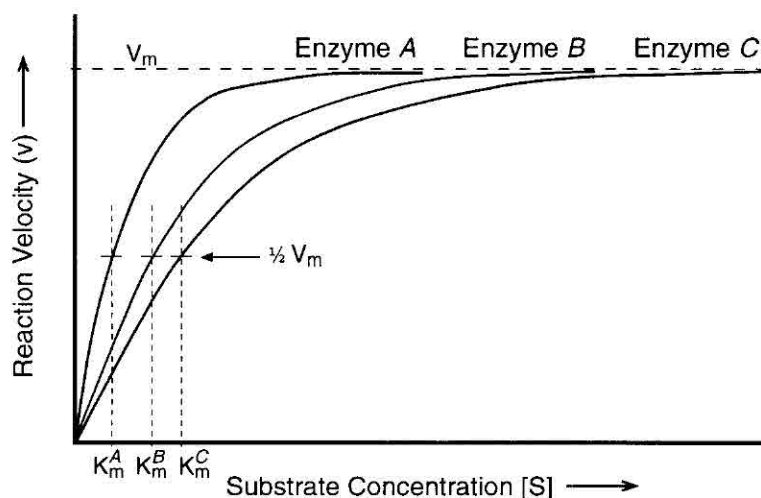
Figure 3.9. Substrate concentration-velocity curves and the corresponding Michaelis-Menten constants (K_m) for enzymes with high (A), medium (B), and low (C) catalytic efficiency. Note: for simplicity, the maximal velocity, V_m, is equal for the three enzymes.

❑   Using the data plotted in Figure 3.7, estimate K_m, expressed in appropriate units. To determine K_m from your data, draw a horizontal line from 1/2 V_m on the y-axis to the point where the line intersects your curve. A vertical line from this point on the curve to the X-axis shows the substrate concentration producing a reaction velocity of 1/2 V_m (see Figure 3.8). This is the Michaelis-Menten constant (K_m) of alkaline phosphatase.

K_m=

Effect of Enzyme Concentration on V_m and K_m

How should K_m and V_m vary with enzyme concentration? Since the maximal velocity (V_m) is defined as the velocity reached when all enzyme is bound to substrate or product, we would expect V_m to vary directly with enzyme concentration; the more enzyme present, the greater the maximal velocity. See Figure 3.10. Also the velocity at all substrate levels below that needed to reach V_m should also vary with enzyme concentration. Thus, the substrate concentration-velocity curve will be shifted to the left at higher enzyme concentration and to the right at lower enzyme concentration. Therefore, the estimated K_m will be the same for a specific enzyme at any concentration for that enzyme. That is, a horizontal line from 1/2 V_m on the y-axis will intersect the curve at the same point on the x-axis, the point corresponding to K_m.
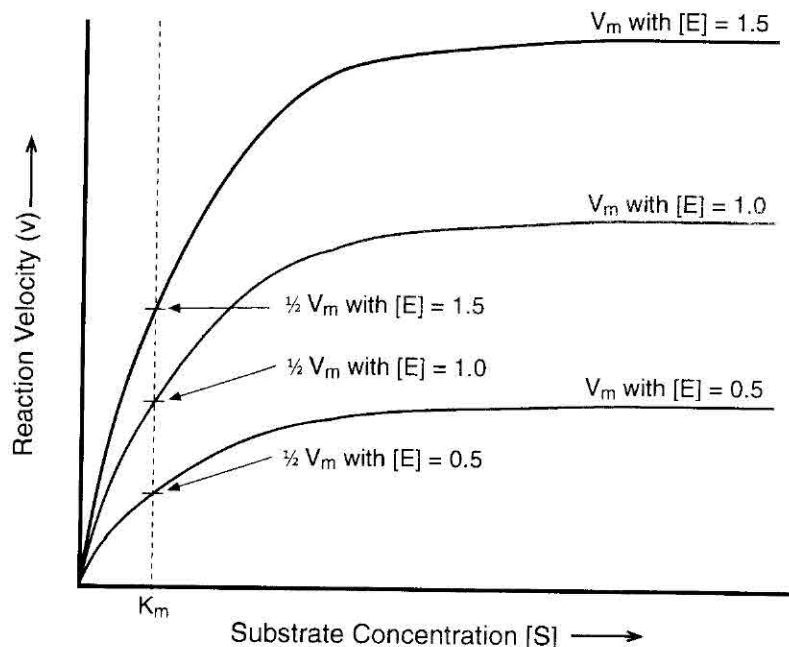
Figure 3.10. Effect of an enzyme concentration of 0.5, 1.0, and 1.5 (arbitrary units) on K_m and V_m for a specific enzyme.

Enzyme Inhibitors

Substrate concentration-velocity curves and estimating V_m and K_m are important tools for studying how certain substances inhibit enzyme activity and for differentiating between **competitive** and **noncompetitive inhibitors**. Generally one series of reactions (each reaction at a different substrate level) are run without the inhibitor and another series are run with it. A competitive inhibitor will produce a substrate concentration-velocity curve with the same V_m but a larger K_m then the curve from the uninhibited reactions, while a noncompetitive inhibitor will produce a curve with a smaller V_m but the same K_m. An explanation for these observations follows.

Generally, competitive inhibitors are structurally similar to the normal substrate of the enzyme and, thus, compete with the substrate for enzyme (Figure 3.11, A and B). Because of their structurally similarity, competitive inhibitors can bind temporarily to the active site of the enzyme but their effect can be overcome by adding more substrate. Thus, the presence of a competitive inhibitor produces a curve with the same V_m, but higher substrate levels are required to reach V_m. This shifts the curve to the right of the uninhibited condition and a larger K_m results. See Figure 3.12.
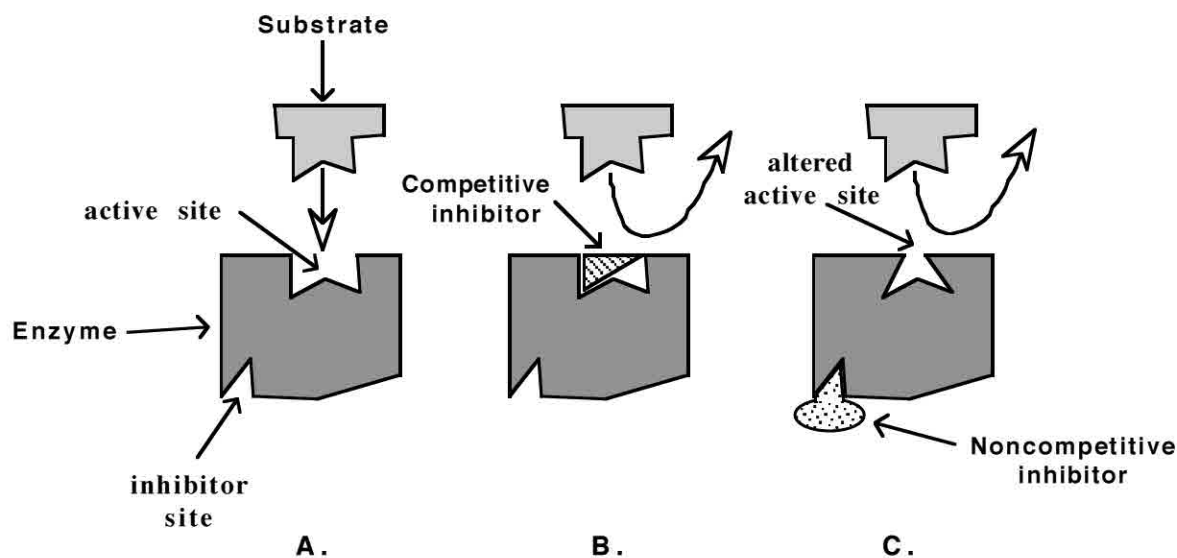
Figure 3.11. Cartoon showing (A.) uninhibited enzyme-substrate interaction, and the action of a competitive (B.) and (C.) noncompetitive inhibitor in disrupting the formation of the enzyme-substrate complex.
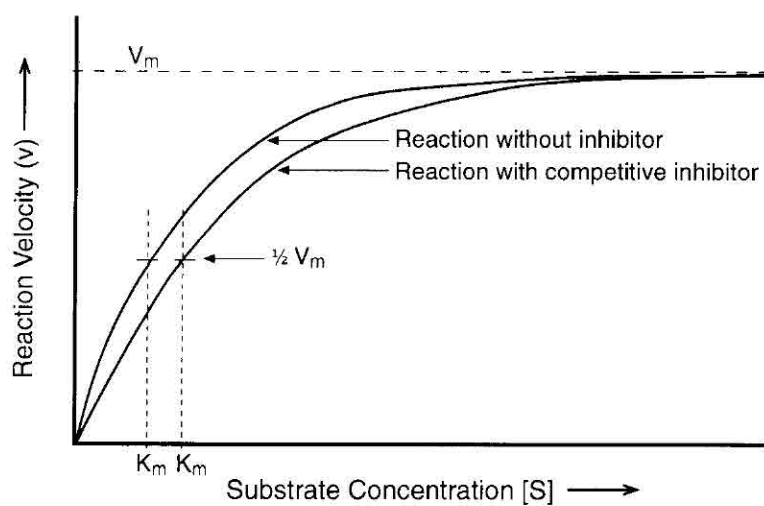
Figure 3.12. Substrate-velocity curves and the estimates of V_m and K_m for an enzyme alone and in the presence of a competitive inhibitor.

A noncompetitive inhibitor binds not at the active site of the enzyme but to another site on the surface of the enzyme. Binding of the inhibitor to its site on the enzyme changes the enzyme's conformation (shape), altering the active site and rendering the enzyme ineffective as a catalyst (Figure 3.11 A and C). Due to the reduction of the number of effective enzyme molecules in the reaction system (those bound to the noncompetitive inhibitor), the V_m of the resulting substrate concentration-velocity curve is less than for the uninhibited condition. However, because the enzyme molecules not bound to inhibitor are able to function at their normal level of catalytic efficiency, the shape of the curve will be normal and the estimated K_m will be the same as the uninhibited condition. See Figure 3.13.
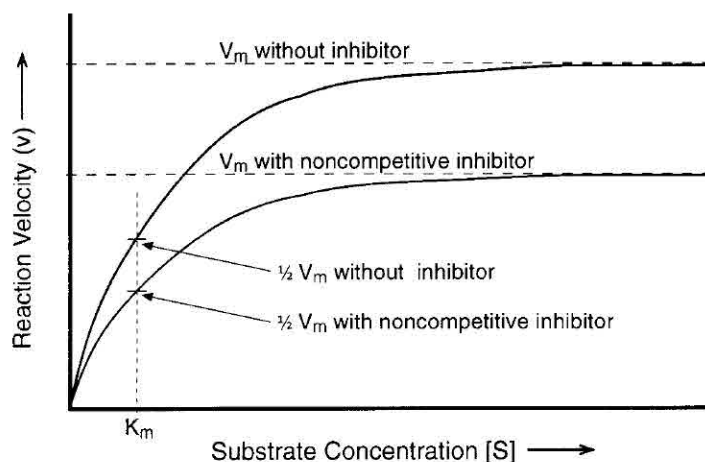
Figure 3.13. Substrate-velocity curves and the estimates of V_m and K_m for an enzyme alone and in the presence of a noncompetitive inhibitor.

These and other types of inhibitors are important in the control of cellular metabolism. For example, in some biosynthetic pathways the end product of the pathway is a competitive inhibitor of the enzyme that catalyzes the first reaction step in the pathway. How would this arrangement result in regulation of the pathway to produce the proper amount of product? See p. 157-159 in Campbell and Reece (2008). You will learn more about the importance of enzyme inhibitors later in the course when you study the biochemistry of cellular respiration.

CONCLUDING ACTIVITIES AND DISCUSSION

BE SURE THAT YOU COMPLETE THE FOLLOWING ACTIVITIES AND CAN ANSWER THE QUESTIONS POSED BEFORE YOU LEAVE LAB TODAY.

? Do your data, and those of the other lab groups, support the model for enzyme catalysis? Compare your curve in Figure 3.7 with the curve in Figure 3.3 showing the expected relationship between substrate concentration [S] and reaction velocity (v)?

❑ Using your group's data plotted in Figure 3.7, estimate V_m, expressed in appropriate units. To determine V_m from your data, you need to estimate the reaction velocity reached when the curve becomes horizontal (see Figure 3.8).

$V_m =$

❑ Using your group's data plotted in Figure 3.7, estimate K_m, expressed in appropriate units. To determine K_m from your data, draw a horizontal line from $^1/_2$ V_m on the y-axis to the point where the line intersects your curve. A vertical line from this point on the curve to the X-axis shows the substrate concentration producing a reaction velocity of $^1/_2$ V_m (see Figure 3.8). This is the Michaelis-Menten constant (K_m) of alkaline phosphatase.

$K_m =$

For comparison, examine the following Michaelis-Menten constants of selected enzymes.

Enzyme (and substrate)	**K_m (mole/liter)**
Alkaline phosphatase (*p*-nitrophenyl phosphate)	_____
Carbonic anhydrase (HCO_3^-)	9.0×10^{-3}
Catalase (H_2O_2)	2.5×10^{-2}
Chymotrypsin (N-Acetyltyrosinamide)	3.2×10^{-2}
Glutamate dehydrogenase (Glutamate)	1.2×10^{-4}
Hexokinase (Glucose)	1.5×10^{-4}

? If you ranked these five enzymes and alkaline phosphatase (based on your estimate of its K_m) from most catalytic efficient (1) to least catalytic efficient (6), what ranking would alkaline phosphatase receive?

_____

? Do the class data showing the influence of enzyme concentration on V_m and K_m support the model for enzyme catalysis? Enter the estimated V_m and K_m values from the different lab groups into Table 3.2. What are the expected results based on the model?

Table 3.2. Maximal velocities (V$_m$) and Michaelis-Menten constants (K$_m$) estimated from substrate concentration-velocity curves for reactions run with 0.5, 1.0, 1.5, and 2.0 mg/100 ml alkaline phosphatase, with *p*-nitrophenyl phosphate as substrate.

Enzyme Concentration (mg/100 ml)	V$_m$	K$_m$
0.5		
1.0		
1.5		
2.0		

REFERENCES AND SUGGESTED READINGS

Campbell, NA.; Reece, JB. Biology. 8th ed. San Francisco, CA: Pearson-Benjamin/Cummings; 2008.

Gray, CJ. Enzyme-catalysed reactions. London: Van Nostrand-Reinhold; 1971.

Koshland, DE. Protein shape and biological control. Scientific American 205. San Francisco, CA: W.H. Freeman and Co.; 1973.

Lehninger, AL. Biochemistry. New York: Worth Publishing Co.; 1982.

Stryer, L. Biochemistry. New York: W. H. Freeman and Co.; 1995.

POST-LAB WEB ASSIGNMENT

To test your understanding of this laboratory, complete the associated web activities located in the lab section of the course web site.

URL = http://biog-101-104.bio.cornell.edu/BioG101_104/tutorials/enzymes.html

You will encounter questions similar to these on major quizzes and the practical examination.

NOTES ON ENZYMES

Name   **Section**

DESCRIPTIVE STATISTICS WORKSHEET

To provide you with some experience with descriptive statistical methods, your lab instructor will help you assemble a sample of data to analyze for tomorrow's lab. Record the data in the following table and provide a relevant title for this table (see the Scientific Writing Appendix for information on constructing tables and figures). Using these data, complete the tasks (1–3) listed below the data table.

Table 1.

1. Using information presented on pages A35–A43 of the Statistical Reference Appendix, calculate the mean and standard deviation of the data in Table 1.

2.   Using information presented on pages A41–A43 of the Statistical Reference Appendix, calculate the
median and interquartile range (IQR) of the data in Table 1.

3.   Using information presented on pages A43–A45 of the Statistical Reference Appendix, plot side-by-
side in the same graph (Figure 1 below), the mean and standard deviation bars and a boxplot of
these data. **Provide a relevant title for this figure** (see pages A21–A23 in the Scientific Writing
Appendix for information on constructing tables and figures).

Figure 1.

CHAPTER 4 – OSMOSIS AND MEMBRANE PERMEABILITY

LABORATORY SYNOPSIS

In this study of osmotic phenomena, you will observe osmotic responses typical of plant and animal cells, and osmotic changes induced by permeation of substances into cells. You and your lab mates will estimate the relative permeability of mammalian (bovine) erythrocytes to nine different nonelectrolyte substances, and consider characteristics of these substances which may influence their ability to pass through the erythrocyte cell membrane. Using descriptive statistics and a statistical test, you will compare the data obtained from certain treatment groups to determine if the differences between the rates of permeation of different substances through the erythrocyte membrane are statistically significant.

LEARNING OBJECTIVES

Conceptual

At the end of this laboratory study you should

1. understand the basic principles of **diffusion**, **osmosis** and **water potential**.

2. have observed, and understand in terms of the principles in #1, the osmotic responses of plant and animal cells.

3. have a general understanding of the fluid-mosaic cell membrane model and the functions of transport proteins in this model.

4. understand the similarities and differences between simple diffusion and facilitated diffusion.

5. understand the permeability characteristics of the phospholipid phase of the cell membranes in relation to the properties of various solutes.

6. understand the meaning of the terms hyperosmotic, hyposmotic and isosmotic.

7. know the rationale for the procedures used in the study of erythrocyte membrane permeability.

8. understand the concepts of **population, sample** and **replicate** as they are used in statistics.

9. understand the descriptive statistics **mean** and **standard deviation** and how they can be used in data analysis.

After you have completed the worksheet on statistical testing, you should understand

1. the advantages of a paired investigative design.

2. the basic four step procedure for statistical testing.

3. the **null** and **alternative hypothesis** as expressions of the two possible ways the observed results can be compared.

4. the **alpha level** and the conventional use of .05 and .01 levels for determining statistical significance.

5. the procedure of calculating a **test statistic** and comparing it to a **tabular statistic** to make a conclusion regarding the rejection of a null hypothesis.

READING ASSIGNMENTS

Before coming to this laboratory you should read:

the following sections in Campbell and Reece (2008):

Osmosis: Chapter 7.3, p. 132-135 (ignore isotonic terms).

Transport in Plants: Chapter 36 p. 767-782.

Red Blood Cells: Chapter 42.4 p. 911- 915.

The Introduction section in this chapter, p. 65-68.

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

1.   A.   What is the standard reference system used for determining the water potential of any other aqueous system?

      B.   What is the water potential of the standard reference system?

      C.   Why do the solutes in a solution have a negative effect on the water potential of the solution?

2.   A small piece of potato is composed of cells whose average turgor pressure is +0.3 MPa (megapascal) and average osmotic potential is -0.6 MPa. The potato piece is put in a cup filled with a salt solution whose water potential is -0.5 MPa. Will the potato piece gain or lose water? Why?

3.   Are erythrocytes likely to have a turgor pressure of +0.3 MPa? Why?

4.   What is the osmolarity of a solution which has 0.1 mole of sucrose, 0.2 mole of ethanol and 0.1 mole of NaCl in a total volume of 1.0 liter? (Sucrose and ethanol do not ionize in solution.)

5.   You have three solutions which are isosmolar (have the same osmolarity). Solution A contains only glucose, which does not dissociate in solution. Solution B contains only potassium chloride (KCl) which dissociates completely into K^+ and Cl^- ions in solution. Solution C contains only magnesium chloride ($MgCl_2$) which dissociates completely into Mg^{2+} and Cl^- ions in solution. Which solution has the greatest **molarity**? Why?

<div align="right">

Paul R. Ecklund
Jon C. Glase

Revised June 2010
Mark A. Sarvary

</div>

SCIENTIFIC WRITING ASSIGNMENT

This assignment is a scientific poster and abstract that reports the results of this laboratory. The required format for this poster is described in Appendix 3. The required sections for the full poster included a Title, Introduction, Methods, Results, Discussion, and Literature Cited sections and an individually written abstract. Further instructions will be discussed in lab.

The first draft of the poster text and the abstract must be typed or word-processed and double-spaced in 12 point fonts of a legible typeface with one-inch margins. The text of the paper from title through the literature cited section should not be more than eight pages, excluding tables and/or figures and the appendix. The poster should be a full poster format, printed in Mann library or any other print shop.

The first submission of the poster text (due June 19) should include a title, an introduction, methods, results, a discussion and a literature cited sections. The appropriate use of descriptive statistics in presenting your results is expected, as well as statistical analysis where appropriate. This will be graded and returned to you, and then the full poster with all sections and the abstract will be due June 29. If you plan to print your poster in Mann library, please keep the summer library hours in mind: M-Th 8am-8pm, F 8am-5pm and Sat12pm-6pm.

Because the paper is based on the results of this lab, you should be very conscientious about keeping track of your activities during the lab and recording the data from the experiment. Few things are as frustrating as trying to write up a report of any sort when you haven't taken good records of what happened.

INTRODUCTION

Free Energy

Throughout this discussion the term "system" refers to any part of the world isolated by real or conceptual boundaries from its surroundings. The free energy of a system is the capacity of that system to do useful work. Thus, free energy is potential energy, and the term **potential** is used for various factors which contribute to the free energy of a system. A biological system consists of many components: water, nonelectrolyte solutes, ions, macromolecules, etc. Each component (molecular or ionic species) contributes its **free energy** to the total free energy of the system. In many cases the "work" accomplished by a component is nothing more than the **net** movement of molecules or ions of that component down a free energy **gradient**. The driving force for a component's net movement is a difference in its free energy in two parts of the system, and the rate of its net movement is proportional to the magnitude of the difference. In most biological systems, the concentration of a substance is the major, or only, factor contributing to its free energy. Therefore, the direction and rate of a substance's net movement depends on its concentration gradient.

Water Potential

Water potential is a quantity which is proportional to the free energy of water and is expressed in units of pressure (e.g., mm of Hg, atmospheres, pascals, or bars). The commonly used symbol for water potential is the Greek letter psi (Ψ). The Ψ of a system is an indication of the **relative** concentration of water molecules available for movement in the system. The standard system for comparison is pure water under atmospheric pressure and is arbitrarily given a Ψ equal to zero. Any factor which increases the concentration of water molecules available for movement in an aqueous system compared to that in the standard system has a positive effect on the Ψ of the system (that is, it gives the Ψ of the system a positive value compared to the zero value of the standard system). Conversely, any factor which decreases the concentration of free water molecules in an aqueous system relative to the concentration in the standard system has a negative effect on the Ψ of the system (it gives the Ψ of the system a negative value compared to the zero value of the standard system). The most important factors which determine the Ψ of biological systems are:

1. **Pressure**: According to Pascal's law, an external pressure applied to any confined fluid increases the pressure at every point in the fluid by the amount of the external pressure. An increase in the pressure of an aqueous system pushes water molecules closer together, thereby increasing their concentration (number of molecules per volume unit). However, because water is not readily compressible, the increased pressure "pushes" water molecules away from the region to which it is applied. Therefore, an increase in pressure makes a positive contribution to the Ψ of the system, and, conversely, a decrease in pressure has a negative effect on the Ψ.

2. **Solutes**: Molecules and ions dissolved in an aqueous solution are soluble because they attract and hold water molecules. In doing this they disrupt the orderly arrangement of water molecules. Water

molecules bound to solutes are not as readily mobile as those which are unattached; therefore, solutes have a **negative** effect on the Ψ of an aqueous system. The **solute potential** (also called osmotic potential) of an aqueous solution is an indirect measure of the relative decrease in water molecules available for movement, due to their attraction and binding to solute particles, compared to the amount of readily mobile water molecules in the standard system (pure water) which has no solutes and thus, no solute potential. The magnitude of the solute potential of a solution is directly proportional to the concentration of solute particles (molecules and/or ions) and is given a **negative** value because the concentration of unbound water molecules is reduced.

3.   **Matrix**: This term refers to insoluble particles and surfaces which attract and bind water molecules thereby disrupting their orderly arrangement and reducing their mobility. Like solutes, matric materials have a **negative** effect on the Ψ of the system because they decrease the concentration of unbound water molecules. The matrix of biological systems includes cell walls, insoluble macromolecules, microtubules, microfilaments, insoluble salt crystals and membrane constituents. A major constituent of most soils is a matrix of minute, but insoluble, particles which, under certain conditions, greatly influence the availability of soil water to plant roots. Soil scientists can separate the matric fraction of a soil and estimate the soil's matric potential. Biological systems are not so easily manipulated. Conceptually one can distinguish the solute potential from the matric potential of a cell, but in practice these two components of a cell's water potential are not readily separated and generally they are regarded together as **solute potential**.

The water potential (Ψ) of any aqueous system is the **algebraic** sum of the following:

a.   Pressure on the system <u>minus</u> atmospheric pressure (symbolized by Ψ_p for pressure potential): may be a positive or negative value.

b.   Solute potential (Ψ_s): always a negative value

i.e., $\Psi = \Psi_p + \Psi_s$

Water potential and its components are expressed in units of pressure. Presently, the **megapascal** (MPa) is the preferred pressure unit. One MPa is equal to 10 bars, or approximately 10 atmospheres.

In all cases, water molecules move from a region with a more positive Ψ to a region with a more negative Ψ. Be sure you understand this. Also, one must realize that a region with a Ψ of a relatively small negative value has a more positive Ψ than a region with a Ψ having a relatively large negative value. For example, a region with a Ψ = -0.2 MPa has a more positive Ψ than one with a Ψ = -1.0 MPa. The terms "larger" and "smaller" and their synonyms are inappropriate adjectives to describe Ψ unless one specifies what these relative terms mean. For example, a "greater" Ψ could mean one which is more positive or one with a larger absolute value even though it is negative.

<u>Application of Water Potential Concepts to the Study of Osmosis in Cells</u>

Cells are considerably more complex than the simple systems composed of solutions and semipermeable membranes generally used to explain osmosis. In addition to the plasma (cell) membrane surrounding the cell, numerous membranes are found within the cell's cytoplasm surrounding various vesicles, vacuoles and organelles and composing the extensive endoplasmic reticulum. Collectively, these intracellular membranes, microfilaments, microtubules and insoluble particles within the cytoplasm compose the matrix within the cell and contribute to the solute potential of the cell. Furthermore, the cell's cytosol is not a simple solution with only one or a few solute species; it is a complex solution of numerous kinds of molecules and ions, in various concentrations, all of which function as solute particles and contribute to the cell's solute potential.

Most of the volume of a mature plant cell is a vacuole containing a solution of several ionic and molecular species. Since the thin layer of cytoplasm between the plasma membrane and the vacuolar membrane (tonoplast) occupies so little of the cell's volume, its contribution to the cell's Ψ often is disregarded. Consequently, in plants osmosis often is considered to occur between the region outside the plasma membrane and the vacuole; the plasma membrane, tonoplast and the cytoplasm between these membranes are treated collectively as a single "membrane" through which water passes.

Cells without cell walls cannot develop internal pressures which greatly exceed the pressure of the cell's immediate environment. Such cells can accumulate water by osmosis and enlarge until the plasma membrane is stretched to its maximum surface area; further uptake of water ruptures the plasma membrane. Consequently, pressure in excess of atmospheric pressure is not an important factor in determining the Ψ of cells without walls. Cells possessing cell walls, however, can develop internal hydrostatic pressures which are much greater than the external pressure. As water enters the cell by osmosis, the cell's volume increases, but expansion is restricted by the rigid cell wall. Net movement of water into the cell can occur until the hydrostatic pressure in the confined volume is great enough to counteract equally the solute potential drawing water into the cell. The Ψ of cells with walls is greatly influenced by the hydrostatic pressure produced in them.

Osmolarity

Campbell and Reece (2008) use the terms "isotonic," "hypertonic," and "hypotonic" to describe the relative concentrations of solute particles in solutions. Unfortunately this is an inappropriate use of these terms. We use the more appropriate terms: "isoosmotic," "hyperosmotic" and "hypoosmotic." Physical chemists and many biologists use the term **osmolarity** to express a solution's concentration of solute particles. Recall that molarity expresses the number of moles of a certain substance per liter of solution, and that a mole consists of Avogadro's number (6.023×10^{23}) of molecules. Similarly, osmolarity expresses the number of "moles" of solute particles per liter of solution. For example, a 1.0 osmolar solution has 1.0 "mole" (Avogadro's number) of solute particles per liter. However, unlike molarity, which is expressed in terms of a single molecular species (for example, 1.0 M sucrose, 0.5 M NaCl), osmolarity may be used to express the concentration of a mixture of various molecular and ionic species in solution. That is, it expresses the concentration of all solute particles without regard to the kinds of particles present. The osmolarity of a solution having only one solute which is a non-ionizing substance, such as glucose, is equal to the molarity of the solution because the molecules do not dissociate into ions in solution and each molecule is a solute particle (that is, the number of solute particles = the number of molecules). Electrolytes dissociate into ions when in solution, yielding more than Avogadro's number of solute particles per mole. Salts dissociate completely into ions. Ideally, a mole of NaCl in solution would yield two times Avogadro's number of solute particles (ions). However, at higher concentrations ions interact by electrostatic attraction thereby decreasing the number of solute particles in the solution. For example, a 0.10 M NaCl solution is 0.187 osM (abbreviation for osmolar) instead of the expected 0.20 osM if no ionic interaction occurred.

Differential Permeability of Cell Membranes

One of the main functions of the cell membrane is to regulate selectively what passes into and out of the cell. Molecules may move through a membrane by simply diffusing through the lipid bilayer or by passing through transport proteins which span the membrane. Molecules vary immensely in their ability to move through cell membranes, and certain physical factors seem important in determining this ability. Molecular size is important, since membranes are more permeable to smaller molecules than they are to larger ones, if all other molecular characteristics are similar. Molecules which diffuse through the lipid bilayer must be soluble in the aqueous medium on both sides of the membrane and also soluble in the hydrophobic interior of the membrane. Since solubility in water requires polarity and solubility in a lipid requires a lack of polarity, these molecules must have both polar and nonpolar portions. The ratio of nonpolar to polar portions of a solute's molecules determines the solute's lipid/water **partition**

coefficient, which is the quotient of its solubility in a lipid divided by its solubility in water. In general, a solute's rate of diffusion through a membrane is directly related to its lipid/water partition coefficient. Ions, which carry an electrical charge, in general cannot move freely through membranes; they require transport proteins. We should also recognize that cells of different organisms and cells from different tissues of the same organism vary in the specific permeabilities of their membranes. Usually, these differences can be related to the environment which the organism normally inhabits or to the functional aspects of the cell in question.

In general, water molecules move through a membrane more rapidly than other molecules. This has been difficult to explain theoretically as a simple diffusion phenomenon because the strong polarity of water molecules should impede their movement through the hydrophobic interior of a membrane. Evidence for water-specific transport proteins in various biological membranes has been accumulating during the past few years. These transport proteins, called aquaporins, seem to function by facilitating diffusion of water molecules through membranes (see p. 131, Campbell and Reece, 2008).

The major study you and your lab mates will do in this laboratory is a determination of the differential permeability of the cell membrane of mammalian erythrocytes (red blood cells) to certain kinds of molecules. Bovine (cow's) blood will be the source of red blood cells. The following information pertains to mammalian erythrocytes and procedures you will use.

Structure and Function of the Mammalian Erythrocyte

Erythrocytes, the chief cellular component of blood, number nearly six million per cubic millimeter in humans. The main function of the pigment **hemoglobin**, from which erythrocytes derive their red color, is to transport oxygen from the gas exchange surface (gills, lungs, etc.) to the tissues. Hemoglobin also helps carry a waste product, carbon dioxide (CO_2), from the tissues to the respiratory surfaces for excretion. The erythrocytes of most mammals have become so extremely specialized for oxygen transport that they lose their nuclei at maturity. This permits maximum hemoglobin content per cell. In surface view, the cell is round with a diameter of about 8 micrometers (0.008 mm), while in side view it appears as a biconcave disc. When an erythrocyte is placed in an environment whose ψ is more negative than that of the cell, it shrinks and assumes the shape of a sphere with small spikes on its surface. The shrinking of red blood cells is called **crenation**. On the other hand, when an erythrocyte is put in a solution whose ψ is more positive than the erythrocyte's, the cell swells. If it swells beyond the capacity of the cell membrane, the membrane starts to rupture and releases hemoglobin. **Hemolysis** is the rupturing of the erythrocyte membrane and the consequential escape of hemoglobin from the cell.

Using Hemolysis to Measure the Differential Permeability of the Erythrocyte Membrane

One method of measuring the erythrocyte cell membrane's permeability for a substance is to place cells in a solution of the substance and measure the time until hemolysis occurs. Initially the ψ of the test solution should be the same as the ψ of the cells, so that water does not move into or out of the cells because of an initial ψ gradient. The substance will diffuse from the solution, where its concentration is higher, into the cells, where its concentration is lower. As the substance enters the cells, the osmolarity of the cells' contents increases because of the addition of solute particles (the substance's molecules or ions). Consequently, the ψ of the cells decreases, causing water to move osmotically into the cells in direct proportion to the substance's rate of penetration. The uptake of test substance particles and water increases the volume of the cells until hemolysis occurs. The period of time between the addition of erythrocytes to a test solution and the visualization of hemolysis (called the **hemolysis time**) is inversely related to the rate at which test substance particles pass through the erythrocytes' membranes.

OSMOTIC BEHAVIOR OF CELLS–PRELIMINARY STUDIES

Plant Cells–*Elodea* Leaf Cells

Elodea is the aquatic vascular plant you observed in the MICROSCOPY, CELLS & TISSUES laboratory. *Elodea* leaves were selected for this study because you can easily observe their cells with a light microscope.

❑ Observe, on the video monitors, a wet mount of an *Elodea* leaf in tap water. The image observed is produced by the 40X objective of the microscope.

You should be able to see cell walls and green chloroplasts randomly distributed in the cytoplasm of each cell. In the case of cells such as these which possess nonliving cell walls, the term **protoplast** is used for that part of the cell within and including the plasma membrane (living protoplasm and contained vacuoles). When the leaf is in tap water, the protoplast of each cell is fully expanded and pressed tightly against the cell wall. Most of each protoplast's volume is a central vacuole filled with fluid (cell sap) confined by the **tonoplast**, or vacuolar membrane. The remainder of the cytoplasm, with its conspicuous chloroplasts and less apparent other organelles and structures, is restricted to a thin outer layer between the tonoplast and the plasma membrane. You cannot see the tonoplast with light microscopy. The cell wall, which is produced by the protoplast, is a relatively rigid structure composed of cellulose and several other polysaccharides. The cell wall is porous and allows most substances to pass through it. The plasma membrane and the tonoplast are differentially permeable membranes which function as selective filters through which substances must pass to enter and leave the cytoplasm and vacuole. The cell sap in the vacuole is mostly water with a variety of dissolved substances in it. When the cell's vacuole contains all the fluid it can hold, the cell appears "plump" or **turgid**, and the cell wall and cell membrane cannot be distinguished because the cell membrane is pressed tightly against the cell wall. Plant cell vacuoles are not contractile.

❑ In the left half of the space provided below make a sketch of one or more turgid *Elodea* leaf cells showing the distribution of chloroplasts.

❑ Answer the following questions.

1. Is the Ψ_s of the *Elodea* cells more positive than, more negative than, or equal to the Ψ_s of the tap water? Explain.

2. Is the Ψ_s of the tap water more positive than, more negative than, or equal to the Ψ of the tap water? Explain.

3. At this time, is the Ψ of the *Elodea* cells more positive than, more negative than, or equal to the Ψ of the tap water? Explain your answer with respect to your answers to questions 1 and 2.

❑   Observe the *Elodea* leaf preparation as the tap water is replaced with a sodium chloride solution.

The exchange of liquids is accomplished without removing the coverslip by placing a few drops of 0.5 M sodium chloride along one edge and a piece of paper towel along the opposite edge of the coverslip. The paper towel removes water under the coverslip, causing the sodium chloride solution along the opposite edge to move in beneath the coverslip.

❑   Look for a clumping of chloroplasts and gaps between the cytoplasm and the cell wall.

The phenomenon you see is called **plasmolysis**–the shrinking of a protoplast away from its wall due to the loss of water from the cell. Although you cannot see the plasma membrane per se, you can see the outer limit of the cytoplasm where the cell membrane occurs.

❑   Beside your first sketch make a sketch of a plasmolyzed *Elodea* leaf cell showing the distribution of cytoplasm and chloroplasts.

❑   Answer the following questions.

4.   How are the Ψ_s and Ψ_p of the *Elodea* cells changing as the cells lose water?

5.   Write an equation which shows the relationship among the water potentials and solute potentials of the sodium chloride solution and the *Elodea* cells <u>when the cells stop losing water</u>.

6.   What occupies the space between the protoplasts and the cell walls of the plasmolysed cells?

?   Is plasmolysis a reversible process? How could we quickly determine if it is?

<u>Interpretation of Osmotic Behavior of *Elodea* Leaf Cells</u>:

First consider the leaf cells in tap water. Since the cells' cytoplasm contains significantly higher concentrations of solute particles (organic compounds, salts, etc.) than the external medium, the cells' Ψ is more negative than the tap water's Ψ. Thus, water tends to enter the cells osmotically. However, the rigid cell wall normally prevents the cell from enlarging beyond a certain volume, leading to the development of hydrostatic pressure called **turgor pressure**. This is a good example of the interaction between pressure and concentration gradients in determining the net movement of water. All plant cells in an environment with a Ψ less negative than theirs show this turgidity. The tendency of a plant cell to take in water is determined by the difference between the Ψ of the cell and that of its environment. The Ψ of the cell depends on the cell's turgor pressure and solute potential:

Ψ of the cell = turgor pressure + solute potential
            (a positive value) (a negative value)

Water will move into the cell until the Ψ of the cell equals the Ψ of the environment. If a plant cell is placed in an environment with a more negative Ψ than its own, the cytoplasm loses water. If the cell membrane remains attached to the cell wall, a negative pressure (tension) develops within the cell as water is lost; however, in most cases the cell membrane pulls away from the wall as the vacuole and cytoplasm shrink, and the Ψ of the cell is determined by its solute potential.

Most of the living cells in any plant are similar in their general structure to those of *Elodea* leaf; each has a cell wall, a large vacuole, and a thin layer of viscous cytoplasm between the vacuole and the cell wall. The cell wall of most plant cells is quite permeable to water molecules. Although the wall is a relatively rigid structure, it has some elasticity and is capable of being stretched. Consequently, the volume of the cell changes as it gains or loses water.

Animal Cells–Mammalian Erythrocytes (Red Blood Cells)

Visual Determination of Hemolysis (A Demonstration)

Your lab instructor will add four drops of whole bovine blood to each of three test tubes containing 10 ml of different liquids, and will quickly mix the blood with the liquid. Two of the tubes contain 0.3 osM solutions of nonelectrolytes; the Ψ of these solutions is equal to the Ψ of the erythrocytes' cytoplasm.

❑ Observe the result of mixing blood with each liquid and record the result in Table 4.1.

Table 4.1. Results of mixing bovine blood with three liquids.

Liquid	0.3 M mannitol	distilled water	0.3 M propanol
Structural formula of solute	H H H H H O O H | | | | | | HO–C–C–C–C–C–C–OH | | | | | | H O O H H H H H	no solute	H H H | | | H–C–C–C–OH | | | H H H
Observed result			

❑ Explain the observed result of mixing blood with each of the three liquids.

Be certain you understand why the hemolysis of erythrocytes placed in an isoosmotic solution of a substance shows the erythrocyte cell membrane's permeability to that substance. This principle is the basis for the method of measuring membrane permeability in the following study on erythrocyte permeability characteristics.

Erythrocyte Permeability Characteristics

This study is designed to test the hypothesis that the physical characteristics of molecules affect their ability to permeate cell membranes. Specifically, you will examine the effect of molecular size and relative lipid solubility of molecular species on their ability to pass through mammalian erythrocyte membranes.

Hemolysis Time

A method for studying the permeability of erythrocytes to a certain substance is to place blood into a solution of the substance <u>having the same Ψ as that of the erythrocytes</u> and record the time required to reach a specified degree of hemolysis.

A simple yet accurate approach is to mix in a test tube a given amount of blood with an isoomotic solution of the test substance. The test tube is held in front of a slit-lamp to measure the hemolysis time. The slit-lamp consists of a diffuse light source positioned behind a piece of cardboard with a hole in which a black thread is suspended. One holds the tube containing the blood and test solution next to the thread so the light illuminates the tube. At the instant when one can first clearly see the thread, approximately 75% of the cells are hemolyzed. **(Hemolysis time is the time interval after adding blood to the test solution until the thread is seen through the mixture.)**

Procedure

Working in pairs, one person will prepare the test mixtures and the other person will measure the hemolysis times of erythrocytes in 9 different test solutions.

1.   Pair yourself with another person and obtain a test tube rack which you share. (If your lab section has an odd number of students and you have no partner, obtain a test tube rack for your self.)

2.   Put masking tape labels on the tops of 9 test tubes and number them 1 through 9. (These numbers correspond to the nine nonelectrolyte solutions listed in Table 4.2.)

3.   Refer to Table 4.2 to see which number corresponds to which solution. Use an adjustable pipettor to dispense **4.0 ml** of the appropriate solution into each test tube with the corresponding number (e.g., glycerol into tube number 1; methanol into tube number 5; etc.) <u>Please do not use the same pipette tip in different solutions</u>.

4.   Each pair of students obtains:

> 1 adjustable pipettor with a new pipette tip
> 1 stopwatch
> 1 container with erythrocyte stock suspension (2% whole blood)
> 2 small squares of parafilm

5.   Adjust the pipettor to deliver **1.0 ml**.

NOTE: Hemolysis times in most solutions will vary from a few seconds to about two minutes, so the sample must be watched continuously by the observer. However, in glycerol the hemolysis time is around 20 minutes or longer; therefore, prepare the glycerol test sample first and observe it every 5 minutes for 20 minutes, and then at one-minute intervals. <u>Use the lab clock to time this test</u>, so you can test other solutions while you are waiting for it.

The hemolysis times in some of the test solutions are so short that they may not be accurately measured if you do not know exactly what to look for. Therefore, we suggest that you start the stopwatch-timed tests with <u>ethylene glycol</u>. After testing it, you may test the other solutions in any order you wish.

<u>It is extremely important that the same person measures hemolysis time in all 9 test solutions</u>.

6. Working in pairs do the following:

Student A: <u>Thoroughly mix</u> the 2% bovine erythrocyte suspension by gently swirling the flask for a few seconds (<u>do not shake the flask</u>), then pipette 1.0 ml of the suspension into a test solution.

Student B: Start the stopwatch.

Cap the test tube with a piece of parafilm; seal it to the tube with your thumb and immediately invert the tube twice to mix its contents.

Observe the tube's contents through the slit lamp until the thread is seen; immediately stop the watch.

Student A: Record the hemolysis time for the test solution in Table 4.2 to the nearest 100th of a second.

7. Repeat step 6 until student B has measured hemolysis time in all 9 test solutions.

<u>Remember to mix the erythrocyte suspension thoroughly each time before it is added to a test solution</u>.

8. Clean up:

a. Remove labels from test tubes. Wash the tubes in soapy water; rinse them in tap water and then distilled water three times; put them on drain pegs.

b. Eject the pipette tip and discard it in the labeled container.

❑ Record your data in a column of Table 4.2 on the overhead transparency, then record the data from all other pairs in Table 4.2.

Explanations of Parameters Used in Table 4.3

<u>Molar Volume</u>: Sizes of test substance molecules are directly proportional to their van der Waals volumes, expressed as cm^3/mole of substance. The volume of an individual molecule is the van der Waal's volume divided by Avogadro's number (the number of molecules in a mole). Values were obtained from Stein (1986) and from the data of Bondi (1964).

<u>Lipid/Water Partition Coefficient</u>: The relative solubility of a substance in a lipid compared to its solubility in water is given by its lipid/water partition coefficient. The values given in Table 4.3 were obtained using olive oil, which is similar in chemical composition to the lipid portion of biological membranes. To determine the partition coefficient of a substance, the substance is added to water and oil in a container and thoroughly shaken. The mixture is allowed to come to rest, the aqueous and oil phases are separated from each other and the concentration of the substance in each phase is determined.

$$\text{Lipid/Water Partition Coefficient} = \frac{\text{concentration of substance in oil}}{\text{concentration of substance in water}}$$

Table 4.2.   Hemolysis times for bovine erythrocytes in 0.3 M solutions of selected nonelectrolytes.

Nonelectrolyte	Hemolysis Time (sec) *(Group member's data)*										Mean (± S)
	1	2	3	4	5	6	7	8	9	10	
1. Glycerol											
2. Ethylene Glycol											
3. Diethylene Glycol											
4. Triethylene Glycol											
5. Methanol											
6. Ethanol											
7. Propanol											
8. Urea											
9. Thiourea											

Table 4.3. Two-dimensional structural formulas, molar volumes and lipid/water partition coefficients of nine nonelectrolyte substances and mean hemolysis times for bovine erythrocytes in 0.3 M solutions of these nonelectrolytes.

Nonelectrolyte	Molar Volume (cm^3/mole)	Lipid/Water Partition coefficient	Hemolysis Time Mean (± S)

Glycerol
    H
  H O H
H-O-C-C-C-O-H
   H H H
	51.4	7.0×10^{-5}	

Ethylene Glycol
  H H
H-O-C-C-O-H
  H H
	36.5	4.9×10^{-4}	

Diethylene Glycol
  H H   H H
H-O-C-C-O-C-C-O-H
  H H   H H
	62	*	

Triethylene Glycol
  H H H H H H
H-O-C-C-O-C-C-O-C-C-O-H
  H H H H H H
	87	*	

Methanol
  H
H-C-O-H
  H
	21.7	9.5×10^{-3}	

Ethanol
  H H
H-C-C-O-H
  H H
	31.9	3.6×10^{-2}	

Propanol
  H H H
H-C-C-C-O-H
  H H H
	42.2	1.4×10^{-1}	

Urea O H ‖ H H-N-C-N-H	32.6	1.5×10^{-4}	
Thiourea S H ‖ H H-N-C-N-H	39.5	1.2×10^{-3}	

* unknown but similar to ethylene glycol

Interpretation of Results: Questions to Consider

1.   In this study you and your lab mates made measurements on subsets (**samples**) of "things" from a larger population of the same "things." In this study:

    A.   What is the **population**?

    B.   What constitutes a **sample**?

    C.   What is a **replicate**?

2.   A **parameter** is a quantifiable characteristic of a population of things.

    A **statistic** is a single value or datum of a characteristic obtained from a replicate of a sample, or it is a quantity that is computed from a sample (for example, the mean of a sample).

    In general, entire populations and their parameters cannot be measured directly. Instead parameters are estimated by drawing samples and calculating statistics.

$$\text{Population} \xrightarrow{\text{described by}} \text{Parameter}$$

used to make inferences about

$$\text{Sample} \xrightarrow{\text{described by}} \text{Statistics}$$
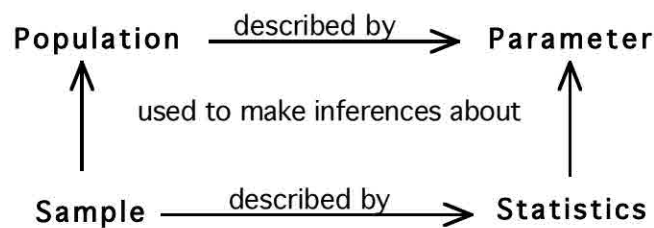
    A.   What parameter was <u>directly</u> estimated in this study? Give a complete and precise answer.

    B.   What characteristic of the population was <u>indirectly</u> estimated in this study?

    C.   What is the relationship between these two characteristics?

3.   Assuming that the three glycols tested have similar partition coefficients, does molecular size appear to influence the rate at which the three kinds of glycols move through the erythrocyte membrane? Explain your answer.

4.   A.   Compare the mean hemolysis times and their standard deviations for erythrocytes in methanol, ethanol and propanol. Are the hemolysis times in these three different substances likely to be significantly different from each other? Explain your answer.

     B.   How can you account for the similar hemolysis times in these three solutions?

5.   A.   Thiourea and propanol molecules are about the same size, but these two substances have very different partition coefficients. Which substance would you predict to diffuse into the erythrocytes more rapidly?
          _____

     B.   Do the data support your prediction?

6.   A.   Ethanol and urea molecules are nearly the same size, but the partition coefficients of these two substances are quite different. Which substance would you predict to diffuse into erythrocytes more rapidly?
          _____

     B.   Do the data support your prediction? If not, what explanation can you offer for the results obtained?

7.   A.   Compare the molecular sizes and partition coefficients of thiourea and ethylene glycol. What do you predict about their relative rates of movement through the erythrocyte membrane?

     B.   Do the data support your prediction? If not, what explanation can you offer?

8.   Why do glycerol molecules move so slowly through the erythrocyte membrane?

STATISTICAL TESTING

In the process of completing this worksheet you will be performing statistical tests to determine whether or not the hemolysis times of erythrocytes that received two different treatments are significantly different. Before continuing read pages A43-A44 in the Statistical Reference appendix (Appendix 6).

Following the flow diagram on page A45, note that you will be testing for **differences between groups of data**. There is no control group in the tests you will do on this worksheet. You will be comparing groups of data from two different treatments.

TESTING PAIRED AND UNPAIRED DATA

The data used in the testing procedure may be paired or unpaired depending on how they are obtained. The purpose of this exercise is to show how collecting paired data reduces extraneous variation in the samples and, in turn, how this affects the statistical testing.

Following the testing flow diagram on page A45, the **Wilcoxon signed rank test** is appropriate for testing paired, quantitative data. Read the description of this test and the example of its use on pages A48-A50. Note in the example that H_o can be rejected at $\alpha = 0.05$; therefore, one can conclude that the measured activity of the shrimp in dim light is significantly different from their activity in bright light.

Suppose the same kind of study was done in a way that did not result in data paired by the individual organisms. For example: Ten brine shrimp were individually tested in dim light and placed in a common container after testing. Then each shrimp was individually tested in bright light. Grouping the shrimp after the first treatment precluded obtaining data that can be paired by the organism. Since the data are unpaired, the **rank sum (Mann-Whitney) test** is appropriate to compare the data sets from the two treatments (see pages A50-A51).

Read the description of the rank sum test and the example of its use on pages A50-A52.

2.   Use the **rank sum test** to statistically compare the activity data of brine shrimp in dim light and bright light in the table below. Note that this table contains in the dim light and bright light columns the same data that are in the example of the Wilcoxon signed rank test on page A49, but the data are not paired by the organism. Assign ranks 1 through 20 to the 20 data values.

	Activity (seconds swimming) in:		
Rank	**Dim Light**	**Bright Light**	**Rank**
	68	220	
	106	165	
	200	60	
	44	70	
	155	116	
	70	76	
	180	212	
	90	91	
	60	120	
	63	122	

$T_1 =$ $T_2 =$

$n_1 = 10$ $n_2 = 10$

Test statistic = _____

State the null (H_o) and alternative (H_a) hypothesis for this test:

H_o:

H_a:

Based on n_1 and n_2 and alpha (α) = 0.05, what is the appropriate tabular statistic for this test? Use the 5 column in the appropriate n_1 column of Table 6 on page A52 in the Statistical Reference appendix.

_____

Based on comparison of test and tabular values, will you **reject** or **not reject** H_o? (circle answer)

Based on your decision regarding H_o, what do you conclude about the activity (seconds swimming) of brine shrimp in dim light and bright light?

Treatment effect variation results from differences in the responses of the samples to the different treatments they receive. These between-treatment differences are the types of variation a researcher hopes to measure.

Random variation results from chance occurrences in the sampling and measuring process. This type of variation may occur within and between treatment groups. For example, one source of random variation within a treatment group in the erythrocyte permeability study might be the subjective determination of the end point of hemolysis by different individuals. One should attempt to minimize random variation in a well designed study.

POST-LAB WEB ASSIGNMENT

To test your understanding of this laboratory, complete the associated web activities located in the lab section of the course web site.

URL = **http://biog-101-104.bio.cornell.edu/BioG101_104/tutorials/osmosis.html**

You will encounter questions similar to these on major quizzes and the practical examination.

REFERENCES AND SUGGESTED READINGS

Bondi, A. Van der Waals volumes and radii. Journal of Physical Chemistry 68:441-451. 1964.

Campbell, NA.; Reece, JB. Biology. 8th ed. San Francisco, CA: Pearson-Benjamin/Cummings; 2008.

Karp, G. Cell biology. New York: McGraw-Hill, Inc. 1979.

Stein, WD. Transport and diffusion across cell membranes. Orlando, FA: Academic Press, Inc. 1986.

Van Holde, KE. Physical biochemistry. Englewood Cliffs, NJ: Prentice-Hall, Inc. 1971.

von Blum, R. Experimental studies of permeability in red blood cells. In: Glase, JC, editor. Tested studies for laboratory teaching. Dubuque, IA: Kendall/Hunt Publishing Co., p. 63-119. 1981.

Name _____

Lab. Instructor _____

OSMOSIS/STATISTICAL TESTING WORKSHEET

1. Plot in Figure 4.1 the **mean hemolysis time with error bars** for erythrocytes in each test solution except glycerol (see page A41 in the STATISTICAL REFERENCE APPENDIX for information on constructing error bars). **Provide an appropriate caption for the figure** and completely label all graph axes. See pages A21-A23 for information on figure captions.
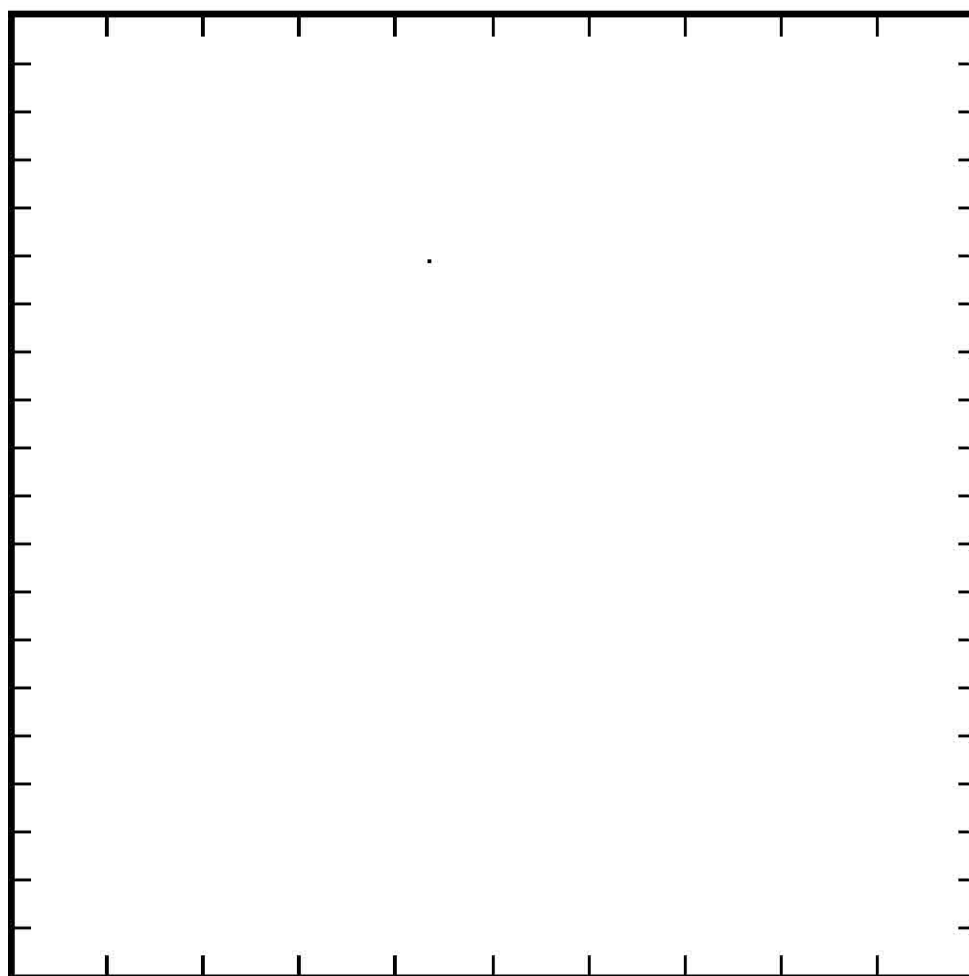
Figure 4.1

In any study in which two or more treatment groups are being compared, two types of variation may occur in the events or objects being measured.

Treatment effect variation results from differences in the responses of the samples to the different treatments they receive. These between-treatment differences are the types of variation a researcher hopes to measure.

Random variation results from chance occurrences in the sampling and measuring process. This type of variation may occur within and between treatment groups. For example, one source of random variation within a treatment group in the erythrocyte permeability study might be the subjective determination of the end point of hemolysis by different individuals. One should attempt to minimize random variation in a well designed study.

2.   A.   What were the different treatments used in the erythrocyte permeability study?

_____

      B.   What two characteristics of each treatment solute were assumed to cause **treatment effect variation** in this study?

1. _____   2. _____

      C.   What characteristic of the erythrocyte population was <u>directly</u> measured in the erythrocyte samples?

_____

      D.   List three possible sources of **random variation** in the estimation of this parameter.

_____

_____

_____

Your chances of detecting treatment effect variation is improved by using a **paired** investigative design. In a paired design, each datum from a replicate in one treatment group is matched (paired) with a datum from a replicate in another treatment group. The basis for matching the two data is a commonality in the way they were obtained, which presumably eliminates or reduces the effect of one or more sources of random variation. Differences between the data paired in this way are more likely to be the result of the different treatments given to the replicates.

3.   A.   How was this study designed to enable you to pair the data in a meaningful way?

      B.   How would pairing of this type help reduce random variation?

4.   In the space provided below, use the **Wilcoxon signed rank test** to statistically compare the hemolysis times for erythrocytes placed in 0.3 osmolar solutions of **methanol** and **ethanol** paired by observer. (The Wilcoxon signed rank test is covered on pages A47-A49 in the Statistical Reference appendix.)

Pair	Hemolysis Time in Methanol	Ethanol	d	Absolute Value of d	Rank of Absolute Value of d
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

Sum of negative ranks (T-) = _____

Sum of positive ranks (T+) = _____   Test statistic = _____

State the null (H_o) and alternative (H_a) hypothesis for this test:

H_o:

H_a:

Based on the number of pairs compared and alpha (α) = 0.05, what is the appropriate tabular statistic for this test? (see Table 5 on page A49 in the Statistical Reference appendix)

_____

Based on a comparison of test and tabular values, will you **reject** or **not reject** H_o? (circle answer)

Based on your decision regarding H_o, what do you conclude about the hemolysis times of bovine erythrocytes in methanol and ethanol?

5.   A.   In the space provided below, use the Wilcoxon signed rank test to statistically compare the
            hemolysis times for erythrocytes placed in 0.3 osmolar solutions of **ethylene glycol** and **urea**
            paired by observer. Complete all needed information in this table.

Pair	Hemolysis Time in Ethylene glycol	Urea	d	Absolute Value of d	Rank of Absolute Value of d
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

Sum of negative ranks (T-) = _____ Test statistic = _____

Sum of positive ranks (T+) = _____   Tabular Statistic (α =   , n =   ) = _____

therefore (circle),   **reject H$_o$**   **do not reject H$_o$**

      B.   Define alpha level:

      C.   If you rejected the null hypothesis in the test just completed (part A), what is the probability
            that you are <u>correct</u> in doing so?

                                      _____

      D.   Briefly explain your answer to part C.

CHAPTER 5 – PHOTOSYNTHESIS

LABORATORY SYNOPSIS

In this laboratory you will consider the general anatomy of leaves and how that anatomy is related to photosynthesis and gas exchange by leaves. Then you will conduct a study to determine the effect of light intensity on the net relative rate of photosynthesis by small discs cut from spinach leaves.

LEARNING OBJECTIVES

Conceptual

1. Understand the meanings of gross photosynthesis and net photosynthesis and how they differ. And be able to describe the photosynthetic reaction and factors that influence its rate.

2. Understand the general anatomy of a leaf and know which cell types are photosynthetic and which are not.

3. Understand the function of the bicarbonate solution in the study of photosynthesis of leaf discs.

4. Understand how the vacuum infiltration procedure causes leaf discs to sink, and how photosynthesis causes them to float.

5. Understand the inverse relationship between time to float and rate of photosynthesis and why the reciprocal of time to float can be used as an estimate of the relative rate of photosynthesis of leaf discs.

6. Understand the limitations of using the liquid infiltration - time to float procedure for estimating relative rates of photosynthesis of leaf discs.

7. Understand the concept of photosynthetically active radiation (PAR) and the units of light intensity measured by the quantum meter.

8. Know the general photosynthetic reaction and be able to discuss several factors that influence its rate.

9. Understand how fluorescence of chlorophyll a occurs when a solution of photosynthetic pigments is illuminated.

READING ASSIGNMENTS

In Campbell and Reece (2008)

       PHOTOSYNTHESIS: Chapter 10.   p. 185-203

In addition to reading this lab chapter read:

       APPENDIX 4:   SPEARMAN RANK CORRELATION TEST   p. A52-A53

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

1.   The leaf discs used in your study should have the highest proportion of photosynthetic cells that is practical to obtain. To obtain discs with this characteristic, why should you <u>not</u> cut them from leaf regions with large vascular bundles (veins)?

2.   Why do freshly cut leaf discs float when they are placed in an aqueous solution?

3.   Why do leaf discs sink in an aqueous solution after they are subjected to a few treatments of reduced pressure followed by normal pressure (i.e., making and breaking a vacuum)?

4.   Why do sunken leaf discs float after being illuminated in a dilute sodium bicarbonate solution?

5.   Why is dilute sodium bicarbonate, instead of just water, used for the sinking and illumination of leaf discs?

6.   What is the relationship between the photosynthetic rate of liquid infiltrated leaf discs and their time to float when they are illuminated?

7.   In a study sample of 50 data values was obtained. The **mean** of the 50 values was 70 units and the **standard deviation** was $\pm$11 units.

    A.   How many of the 50 data values would you expect to be in the range from 59 to 81 units?

    B.   How many of the 50 data values would you expect to be in the range from 70 to 92 units? (Give the nearest whole number for your answer.)

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Revised May 2010
Mark A. Sarvary
Scott T. Meissner

GENERAL INTRODUCTION

The overall process of photosynthesis in organisms having chlorophyll *a* can be expressed in the following way:

$$CO_2 + 2H_2O^* + \text{light energy} \xrightarrow{\text{chlorophyll } a} O_2^* + H_2O + (CH_2O)$$
$$\text{chemical energy in}$$
$$\text{organic material}$$
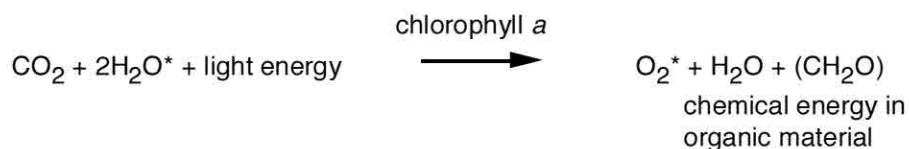
Water is both a reactant and a product of the process. Hydrogen atoms are removed from reactant water, and its oxygen (indicated by the asterisks) forms molecular oxygen, a product. Hydrogen atoms from reactant water reduce CO_2 to form organic molecules (symbolized by CH_2O) and product water. Since carbon dioxide receives the hydrogen atoms removed from water, carbon dioxide must be available so that oxygen production can occur in photosynthesis. Light energy is presented as a reactant because it is transformed into, and stored as, chemical energy in the organic products.

Chloroplasts have several different pigments in (and in some cases <u>on</u>) their membranes. Special forms of **chlorophyll *a*** are the ultimate recipients of the light energy and, with that extra energy, function as catalysts in certain steps of the photosynthetic process. Some of the other pigments initially absorb the light energy and transfer it to the catalytic forms of chlorophyll *a*. However, the main function of some of the yellow pigments in the chloroplast appears to be protection of chlorophyll *a* from destruction by photooxidation in high light intensities.

Many of the cells in a plant are not photosynthetic because they lack chloroplasts and, therefore, cannot obtain energy directly from photosynthesis. Furthermore, photosynthetic plant and algal cells can obtain energy from photosynthesis only when they are in light, yet they need energy all the time. All plant and algal cells, photosynthetic or not, obtain energy by breaking down the organic products of photosynthesis (CH_2O) by a process called respiration. Some of the organic products of photosynthetic cells are transported to non-photosynthetic cells. Respiration can be simply summarized by the following equation:

$$CH_2O + O_2 \longrightarrow CO_2 + H_2O$$

Most plant cells, including the photosynthetic cells, are performing respiration in their mitochondria <u>even when photosynthesis is occurring</u>. Consequently, some of the oxygen molecules produced by photosynthesis are used immediately in respiration in the cells. However, when the light intensity is relatively high, photosynthetic cells produce much more oxygen than they consume in respiration. The excess oxygen first diffuses to other cells and into space between cells in the plant. Eventually much of the excess oxygen diffuses out of the plant into the plant's environment.

When one is measuring the photosynthetic activity of plant tissues it is important to understand that some of the O_2 and organic material produced by photosynthesis is used immediately in **respiration** to supply the plant with needed energy. If one measures the production of oxygen as an estimation of a leaf's photosynthetic activity, s/he is measuring **net photosynthesis**, which is the amount of oxygen produced <u>in excess</u> of that used in concurrent respiration. **Gross photosynthesis** is the total amount of oxygen produced including that used in respiration. **Mitochondrial respiration**, which occurs in leaf tissue whether it is illuminated or not, is often estimated by measuring the consumption of O_2 by the tissue in darkness. The dark (mitochondrial) respiration rate can be used as an estimate of the respiration rate that occurs when the tissue is photosynthesizing. However, in many plant species another form of respiration, called **photorespiration**, occurs in the photosynthesizing tissue when it is illuminated. In

species having significant photorespiration (so called C_3 plants) **net photosynthesis** is the O_2 produced in excess of that consumed by the processes of mitochondrial respiration and photorespiration together. Under the laboratory conditions you will use, the net photosynthesis of leaf pieces is about 70 to 80 percent of the gross photosynthesis.

Both photosynthesis and respiration involve two gases. CO_2 is a reactant and O_2 is a product in photosynthesis; the reverse occurs in respiration. Consequently, one may measure photosynthetic or respiratory activity of biological material by measuring changes in the amount of either of these gases in the immediate environment of the material. Rather sophisticated and expensive equipment is needed to accurately measure <u>directly</u> the amount of O_2 or CO_2 present. In this laboratory you will use simple, inexpensive equipment to indirectly estimate the rate of net photosynthesis of leaf discs.

To fully appreciate the procedure you will use, you need to understand the anatomy of a leaf. Observe Figure 35.17 on p. 750 of Campbell and Reece (2008) and Figure 5.1 These figures show the general anatomy of leaves. Notice that the **epidermis**, the upper and lower surface tissue of the leaf, is composed mainly of cells which fit together tightly. Pores in the epidermis, called **stomata** (sing.; stoma) are formed by pairs of specialized epidermal cells called **guard cells**. Stomata allow exchange of gases between the leaf's interior and the outside environment. Generally the guard cells are the only photosynthetic cells of the epidermis; other epidermal cells lack chloroplasts.
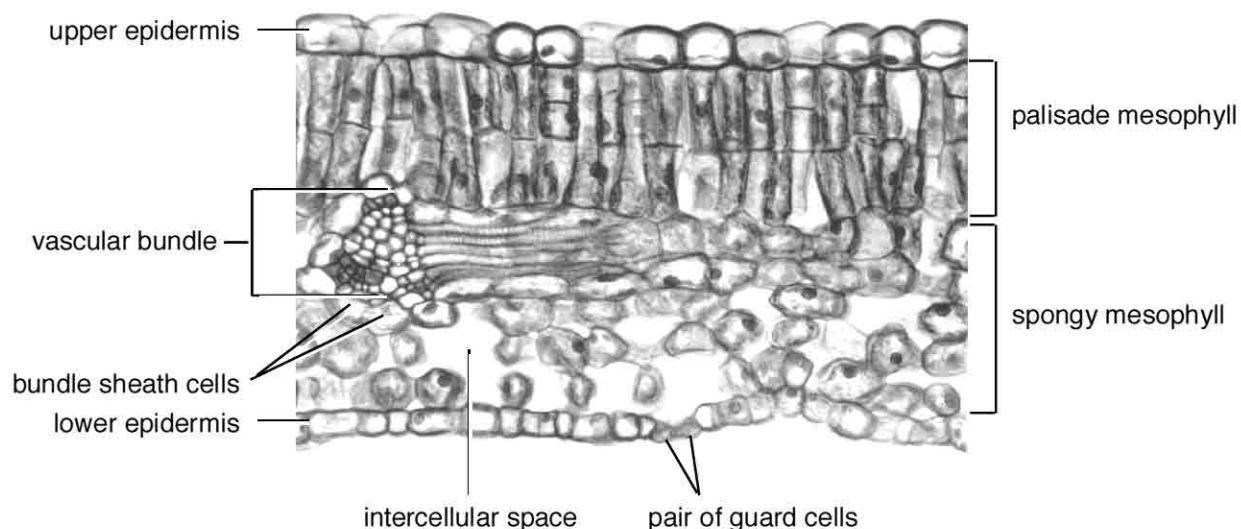
Figure 5.1. Cross-section of a privet (*Ligustrum*) leaf, x430. Privet is a C_3 plant. The vascular bundle runs obliquely to the plane of the leaf section; therefore, both transverse and longitudinal views of the bundle are shown.

The photosynthetic region of the leaf is the **mesophyll** between the two epidermal layers. Leaves of some species have only a **spongy mesophyll** which consists of loosely arranged cells having much space between them. Leaves of many species also have a **palisade mesophyll** composed of cylindrical cells more compactly arranged than those of the spongy mesophyll. The intercellular (between the cells) space of the mesophyll is normally filled with gases including nitrogen, oxygen, carbon dioxide and water vapor (See Figure 10.3, p. 187 in Campbell and Reece, 2008).

Within the leaf is a highly branched vascular system composed of specialized cells joined end-to-end to form tubes. These tubes are part of the continuous vascular system running from the roots through the stem to the leaf of a plant. Some of these tubes carry water and nutrients to the other leaf cells. Other tubes transport carbohydrates, the product of photosynthesis, in solution to other parts of the plant. Cells

of the vascular tissue are not photosynthetic. The conduits of the vascular system are arranged in bundles called **vascular bundles** or veins. Generally each vascular bundle is surrounded by a layer of tightly packed cells called a **bundle sheath**. In many plant species the bundle sheath cells do not have functional chloroplasts. However, in C_4 plants the bundle sheath cells have relatively large chloroplasts and accomplish a part of the overall photosynthetic process which the mesophyll cells cannot do. In the leaves of most plants (even C_4 plants), a supportive tissue composed of nonphotosynthetic cells with heavy cell walls lies between the epidermis and the bundle sheaths of the larger veins (see Figure 10.19, p. 201 in Campbell and Reece, 2008). Consequently, regions of a leaf with larger veins have a smaller proportion of photosynthetic cells than do regions with smaller veins.

In your photosynthesis assay procedures you cut small, uniform discs of leaf material and put them in a solution of sodium bicarbonate (baking soda) in a heavy glass flask. The bicarbonate ions in solution combine with hydrogen ions to undergo the following sequence of reversible reactions, which form CO_2.

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2$$

Thus, bicarbonate ions are the source of CO_2 for photosynthesis. The discs float because gases in the intercellular space make them buoyant. Using a vacuum pump you alternate decreasing and restoring the pressure on the leaf discs and solution in the flask. When the pressure is decreased, gases are removed from the intercellular space. When the initial pressure is restored, the bicarbonate solution is forced into the intercellular space. After a few cycles of making and breaking the vacuum on the leaf discs and solution, enough solution enters the leaf discs to waterlog them and they sink. Then you illuminate the sunken discs in bicarbonate solution. In the presence of light and carbon dioxide, the leaf discs perform photosynthesis. As CO_2 is removed from the solution, the reactions shown above go to the right to maintain a supply of CO_2. Oxygen gas, produced by photosynthesis, diffuses into the intercellular space and displaces the bicarbonate solution. Eventually enough oxygen enters the space to make the leaf discs buoyant and they float. In this procedure the rate of photosynthesis is determined by the amount of oxygen produced per unit time (for example, per minute). However, you cannot actually measure the amount of oxygen produced. Instead you measure the **time to float**, which is the period of light exposure needed to cause a submerged leaf disc to float in the solution. The time to float is <u>inversely</u> related to the rate of photosynthesis in the leaf piece. That is, a relatively long (large) time to float results from a relatively small rate of oxygen production by photosynthesis. The inverse relationship between rate of photosynthesis and time to float can be expressed mathematically.

$$\text{rate of photosyn} \propto \frac{1}{\text{time to float}}$$

$$\text{rate of photosyn} = \frac{K}{\text{time to float}}$$

K is a proportionality constant. It is the volume of O_2 required to make a submerged leaf disc buoyant enough to float. Its quantity is unknown.

$$\frac{\text{rate of photosyn}}{K} = \frac{1}{\text{time to float}}$$

Therefore, a value proportional to the rate of photosynthesis can be obtained by calculating the reciprocal of time to float, and the value is expressed per unit of time.

EFFECT OF LIGHT INTENSITY ON THE RELATIVE NET RATE OF PHOTOSYNTHESIS IN SPINACH LEAF DISCS

Overview of the Study

All members of your lab section, working in pairs, will collectively conduct a study to determine the effect of light intensity on the relative net rate of photosynthesis of spinach leaf discs.

Each pair of students will have an illumination apparatus for exposing leaf discs to several light treatments. Samples of leaf discs will be cut with hand-held paper punches from leaves of spinach. Different light intensities will be obtained by using different wattage lamps and different distances between lamps and leaf discs. Light intensities will be measured with a quantum photometer.

The photosynthesis assay procedures to be used in this study were developed by Wickliff and Chasson (1964) with modifications in the apparatus and procedures by Tatina (1986), Juliao and Butcher (1989) and the author. This photosynthesis assay is not usually used for quantitative studies, because measurements of O_2 or CO_2 can be made more accurately using oxygen electrodes or infrared gas analyzers for CO_2 (Walker and Osmond, 1986). Since this course cannot afford to obtain the number of these expensive instruments needed for several lab sections, you will use an assay requiring less expensive equipment.

The correlation of photosynthetic rate on light intensity is well established in the literature, hence the purpose of this study is not to discover new information. Rather, the main purpose of this study is to determine the suitability of the liquid-infiltrated leaf disc assay for quantitatively studying the effect of light intensity on the relative net rate of photosynthesis. A statistical analysis (Spearman Rank Correlation Test) of the data will help you make this determination.

Rationale for the Study

Recall from your study of enzyme function that the rate of a chemical reaction, or a chemical process such as photosynthesis which consists of several chemical reactions, depends on the concentrations of reactants and enzymes. Since light is regarded as a reactant in photosynthesis, the amount (or intensity) of light may influence the rate of the process. Light energy is in discrete units called photons, and its intensity may be quantified as the number of photons striking a defined surface area per unit time (e.g., the number of photons striking 1 m^2 per sec). Each photosynthetic pigment molecule can absorb only one photon at a time. When the light intensity is relatively low, the number of photons striking the pigment molecules is small enough that all the photons can be absorbed and eventually converted to chemical energy. If all the enzymes and other reactants are present in sufficient quantities, light intensity is the limiting factor and determines the rate of photosynthesis. When the light intensity is relatively high, the pigment molecules are receiving more photons than they can absorb and process in a given period of time; the photosynthetic system is saturated with photons. Under conditions of light saturation, increasing the light intensity will not influence the rate of photosynthesis, because some other factor is limiting the rate of the process. In extremely high light intensities, the pigment molecules may be damaged by the excessive photon bombardment, resulting in a lower rate of photosynthesis than would be seen in saturating, but nondamaging light intensities.

The fact that photosynthesis shows saturation for photons can be considered analogous to an enzyme being saturated for its substrates. For instance, consider the following enzyme catalyzed reactions:

$$A + B = C$$

In this case items A and B are both substrates. When B is available in unlimiting amounts then the rate of the production of C should be related to the concentration of A presented to the enzyme. But if an experimenter used too high a concentration of A then the system might only show saturation. In photosynthesis both light and carbon dioxide must be acquired by the cell. To saturate the system with carbon dioxide you will use a buffer solution. But the intensity of light you select is critical. Figure 5.2 shows the results of one student's experiment in which light intensities of over 300 $\mu E/m^2/sec$ were used. Notice that as the light intensity used was increased there was no trend in the change in the mean of the inverse of the times to float (a measure of the rate of photosynthesis), therefore these data do not support a correlation between light intensity and photosynthetic rate under these experimental conditions. However, based just on the data shown in Fig. 5.2, it would be incorrect to conclude that there is never a correlation between light intensity and photosynthetic rates since there are other light intensities which this data set does not cover. Thus in designing your experimental conditions you will need to consider carefully what experiment conditions you wish to use and then limit your conclusions just for those conditions.
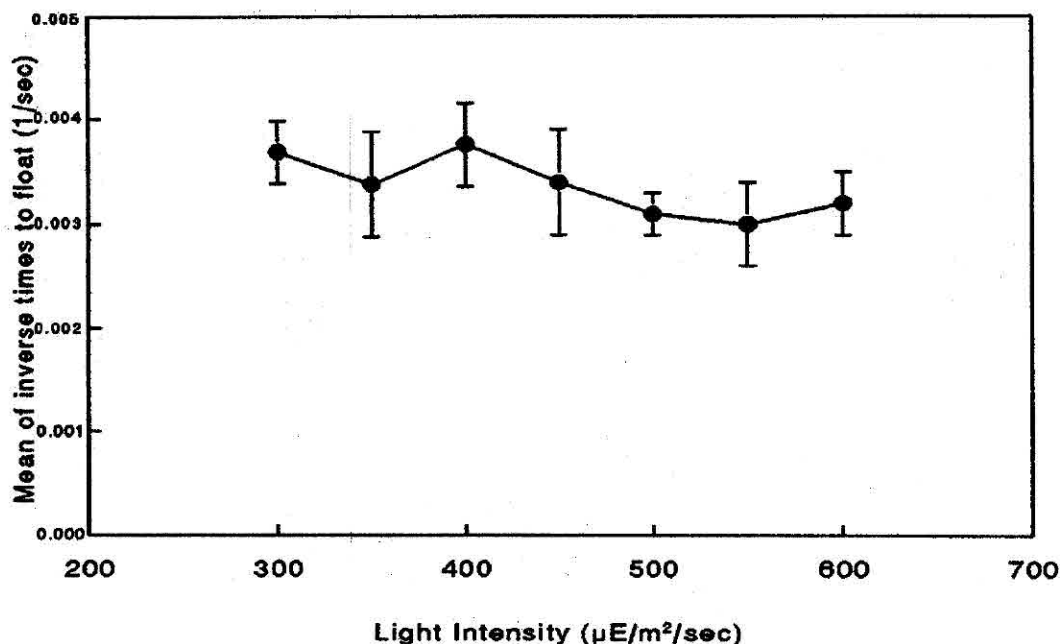
Figure 5.2. Mean of the inverse of times to float for five leaf discs exposed to various light intensities. The error bars represent plus or minus one standard deviation.

The Illumination Apparatus

Figure 5.3 shows the arrangement of the apparatus' components. The wood block beneath the Petri dish containing the leaf discs is essential because it places the Petri dish at the position it will be when the light intensity on the leaf discs is measured.
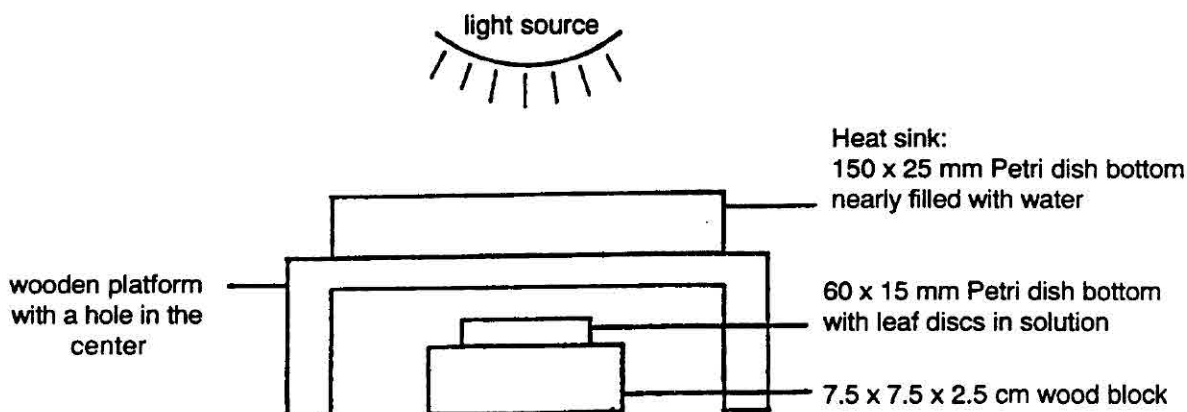
Figure 5.3. Side view of illumination apparatus showing the arrangement of its components.

Design of the Study

The Concept of a Replicate

In your study you will take **samples** from a **population** of spinach leaves. Each sample consists of one or more **replicates**. The statistical definition of a **replicate** is a single trial of an experiment, therefore the number of replicates in an experiment is the equal to the number of trials, or repetitions, of the same treatment in the experiment. Suppose you sink 10 leaf discs in sodium bicarbonate solution, transfer them to a Petri dish, illuminate them and measure their times to float. Is each of the 10 leaf discs a replicate? In this case, no. The 10 leaf discs collectively were given the same single treatment; therefore, the 10 discs are in a single replicate. Another replicate would be another group of 10 leaf discs subjected to the same treatment, including the same concentration of sodium bicarbonate and illumination with the same light intensity.

?   What are the variables being considered in your study?

?   Which one is the independent variable?

?   How might you modify the study to take into account possible leaf-to-leaf variation?

?   What might be the other sources of variations?

In order for the results of a statistical test to be valid, an experiment must be designed to meet the minimum requirements for sample size, pairs of variables, and replicates for the test. Refer to Appendix 4: Statistical Reference and the table below for the minimum data requirements for the Spearman Rank Correlation Test.

Testing for	Statistical test to use	Minimum sample size (no. of replicates)	Minimum no. of pairs of variables
Correlation between different variables	Spearman Rank Correlation Test pp. A52-A53	1	4

PROCEDURE

Equipment and Reagents Needed

Each pair of students needs the following:

1 inoculating loop with the loop bent at a right angle
1 medium scissors
1 stopwatch
1 gooseneck lamp or ringstand with either a 50W bulb, 100W bulb, 150W bulb
1 soft forceps
1 wooden platform with a hole in it
1 wood block with a plastic surface
1 50 ml heavy glass Erlenmeyer flask
1 150 x 25 mm (large) Petri dish bottom
1 60 x 15 mm (small) Petri dish bottom
1 100 x 15 mm (medium) Petri dish (bottom and lid)
A beaker containing about 75 ml sodium bicarbonate solution

NOTE: The bicarbonate solution is 0.01M sodium bicarbonate in a 0.1M phosphate buffer of pH 6.8.

Liquid infiltration apparatuses are attached to laboratory faucets and should be left intact. Each apparatus consists of a rubber stopper around a T-tube connected by tubing to a faucet vacuum pump. Beside each apparatus is a 250 ml plastic beaker; this should remain by the apparatus.

Light intensity is measured with a LI-COR quantum/radiometer/photometer and a quantum sensor which measures **photosynthetically active radiation** (PAR), which is the wavelength range from 400 to 700 nm (nanometers). The light intensity units measured are microeinsteins per square meter per second (expressed $\mu E \ m^{-2} \ sec^{-1}$). An **Einstein** is a mole, or Avogadro's number (6.023×10^{23}), of photons. Therefore, light intensity is expressed as the number of photons striking a surface area of one m^2 each second.

Design of your study

Each pair of students will work with one apparatus and will determine their own experimental design to examine the question of whether or not the rate of photosynthesis correlates with light intensity.

? What is your experimental hypothesis?

? Given the materials available to you how closely can you control experimental conditions?

To explore this use the procedures given below for a trial run of procedures using just ONE leaf disc. Consider the following.

Would all leaf discs sink in the buffer solution equally? Would you expect all leaf discs to float equally? (i.e. how much disc-to-disc variation do you expect?)

What are the maximum and minimum number of leaf discs that could be exposed to each light intensity level? Should you use the same discs or different discs for each treatment?

What range of light intensities can you apply using the materials available?

How much does light intensity vary from location to location in your system?

How precisely can you measure time to float for a leaf disc?

?   Examine the data presented in Table 5.2, what values of light intensity do you choose to use for your study? (Note that to use the Spearman Rank Correlation Test you need at least four different light intensities treatments.)

?   What will constitute one replicate of your study?

Obtaining Samples

1.   Half fill one (or more, if necessary) 100 x 15 cm (medium) Petri dish with sodium bicarbonate solution.

2.   Using a paper punch, punch out 10 discs from one selected spinach leaf. Avoid leaf regions with large veins which have little photosynthetic tissue. Immediately after each disc is cut, put it in the buffer in the Petri dish. Each Petri dish should have discs from only one leaf.

3.   Put about 30 ml of sodium bicarbonate solution in the Erlenmeyer flask.

4.   Use soft forceps or the inoculating loop to transfer the leaf discs into the flask containing bicarbonate solution.

Assembling the Illumination Apparatus

1.   Place the large Petri dish bottom on the wooden platform over the hole and fill the dish nearly to the top with <u>cold</u> tap water. Refer to Figure 5.3 for the arrangement of the components. Be sure you have a lamp with a wattage that is appropriate for the light intensity you are using. Place and adjust the lamp so its reflector will direct the light down through the hole in the platform. <u>Please do not grab the lamp's reflector when making adjustments in its height</u>; the reflector comes off rather easily.

Measuring Light Intensity

1. Fill a small Petri dish 2/3 full with sodium bicarbonate solution and place the dish on the sensor of the quantum radiometer. Carefully move the sensor and dish under the center of the hole in the wooden platform. The sensor is in almost the same location the submerged leaf discs will be in when they are illuminated.

2. Turn on the lamp of your illumination apparatus.

3. Adjust the height of the lamp to give the desired light intensity at the level of the small Petri dish's bottom when it is on the wood block. Measure light intensity in the following way:

    With the LI-COR LI-189 quantum/radiometer/photometer and its quantum sensor:

    Press the ON button. Wait a few seconds for the readout to stabilize. The readout is given in $\mu E\ m^{-2}\ sec^{-1}$.

    NOTE: Use only the ON and OFF buttons (nothing else) on the meter.

4. When you have obtained the desired light intensity with your illumination apparatus, carefully remove the light sensor and petri dish. Since even slight changes in the positions of the lamp and illumination apparatus can cause moderate changes in light intensity, do not move, adjust or bump the illumination apparatus once you have adjusted your set up to the proper intensity. It is best to turn the lamp on and off by plugging and unplugging the electrical cord, rather than using the switch. If you do accidentally move the apparatus, inform your lab instructor.

Liquid Infiltration Procedure

1. Insert the rubber stopper connected to the hose of a vacuum pump into the flask containing your leaf discs. Your leaf discs should be in the bicarbonate solution, not water.

2. Insert the flask all the way into the 250 ml plastic beaker. (This is to protect you if the flask breaks -- very unlikely.)

3. Turn on the cold water valve of the faucet with the vacuum pump. Open the valve all the way. (Be sure the tubing running from the faucet spout is inserted all the way into the sink.)

4. Place a finger over the open end of the tube in the stopper. This closes the system allowing the vacuum pump to decrease the pressure in the flask.

5. Keep your finger on the tube until the solution in the flask boils; allow the solution to boil for about 15 sec., then remove your finger to break the suction. Swirl the contents of the flask to dislodge any discs that may be stuck to the side.
    Note: Merely evacuating the flask will not cause the discs to sink; you must alternate reducing and restoring the pressure in the flask a few times to sink the discs.

6. Repeat steps 4 and 5 several (3-4) times. Remove any discs that have not sunk after this. To the sunken discs, repeat steps 4 and 5 one more time.

7. Turn off the vacuum pump; remove the flask from inside the plastic beaker and remove the stopper from the flask. Please leave the vacuum pump and its attachments intact.

Photosynthesis Assay

1.   Vigorously swirl the contents of the flask to remove the discs from the bottom, and quickly pour the flask's contents into a medium Petri dish. Be sure all the discs come out of the flask.

2.   Pour fresh sodium bicarbonate solution into the <u>small</u> Petri dish until it is 2/3 full.

3.   With soft forceps or an inoculating loop transfer your chosen number of sunken leaf discs to the small Petri dish. As they sink to the bottom of the dish, separate them so they are not overlapping and not next to the edge of the dish.

4.   Place the dish on the wood block and center it under the hole of the platform.

5.   Set the stopwatch to zero. Plug in the lamp and start the stopwatch.

6.   **Note: this step is <u>very</u> important**. Gently and continuously oscillate the wood block under the lamp to prevent the leaf discs from sticking to the bottom of the Petri dish.

7.   If a floating leaf disc covers a submerged disc, use the inoculating loop to move the floating disc.

8.   Note the time to <u>the nearest second</u> when each disc floats to the surface of the liquid. **Some discs float up on edge; regard this also as floating**. Record these times in Table 5.1.

9.   When all the discs in your sample are floating, unplug the lamp.

10. If necessary, repeat the preceding steps for a second replicate or treatment, again using the same number of leaf discs. Record the data in a copy of Table 5.1.

11. Clean up your work area. Dump the contents of all Petri dishes down the sink; thoroughly rinse all plastic and glassware, and invert them on the drain areas.

REFERENCES AND SUGGESTED READINGS

Campbell, NA.; Reece, JB. Biology. 8th ed. San Francisco, CA: Pearson-Benjamin/Cummings; 2008.

Juliao, F.; Butcher, HC. Further improvements to the Steucek & Hill assay of photosynthesis. American Biology Teacher 51:174-176; 1989.

Tatina, RE. Improvements to the Steucek and Hill assay of photosynthesis. American Biology Teacher 48: 364-366; 1986.

Walker, DA.; Osmund, CB. Measurement of photosynthesis <u>in vivo</u> with a leaf disc electrode: correlations between light dependence steady-state photosynthetic O_2 evolution and chlorophyll a fluorescence transients. Proceedings of the Royal Society (London). B:227:267-280; 1986.

Wickliff, JL.; Chasson, RM. Measurement of photosynthesis in plant tissues using bicarbonate solutions. BioScience 14:32-33; 1964.

Table 5.1. Data for leaf discs in one replicate.

Lamp type bulb _____	Time to float for each disc of		Reciprocal time to float	Mean time to float for replicate:
flood _____	min : sec	= sec	per second	_____ sec
Wattage: _____				Mean of reciprocal of time to float:
				_____/sec
Distance between lamp and bench top: _____				
				Standard deviation of reciprocal of time to float:
Light intensity used:				_____/sec
_____μE m^{-2} sec^{-1}				
	Total			

Lamp type bulb _____	Time to float for each disc of		Reciprocal time to float	Mean time to float for replicate:
flood _____	min : sec	= sec	per second	_____ sec
Wattage: _____				Mean of reciprocal of time to float:
				_____/sec
Distance between lamp and bench top: _____				
				Standard deviation of reciprocal of time to float:
Light intensity used:				_____/sec
_____μE m^{-2} sec^{-1}				
	Total			

Lamp type bulb _____	Time to float for each disc of		Reciprocal time to float	Mean time to float for replicate:
flood _____	min : sec	= sec	per second	_____ sec
Wattage: _____				Mean of reciprocal of time to float:
				_____/sec
Distance between lamp and bench top: _____				
				Standard deviation of reciprocal of time to float:
Light intensity used:				
				_____/sec
_____μE m^{-2} sec^{-1}				
	Total			

Lamp type bulb _____	Time to float for each disc of		Reciprocal time to float	Mean time to float for replicate:
flood _____	min : sec	= sec	per second	_____ sec
Wattage: _____				Mean of reciprocal of time to float:
				_____/sec
Distance between lamp and bench top: _____				
				Standard deviation of reciprocal of time to float:
Light intensity used:				
				_____/sec
_____μE m^{-2} sec^{-1}				
	Total			

PHOTOSYNTHESIS WORKSHEET

Name: _____ **Section:** _____

Table 5.2. Class data from correlative study of light intensity and relative net rate of photosynthesis in spinach leaf discs.

Light Intensity (μE m^{-2} sec^{-1})	Mean time to float (seconds)	Mean of the reciprocal of time to float (per second))	Standard deviation of reciprocal of time to float (per second)
50			
100			
150			
200			
250			
300			
350			
400			

1. Plot in Figure 5.4 the **mean reciprocal of time to float with error bars** of spinach leaf discs at varying light intensities. (See APPENDIX 4: STATISTICAL REFERENCE for information on constructing error bars.) <u>Be sure to label all graph axes</u>.
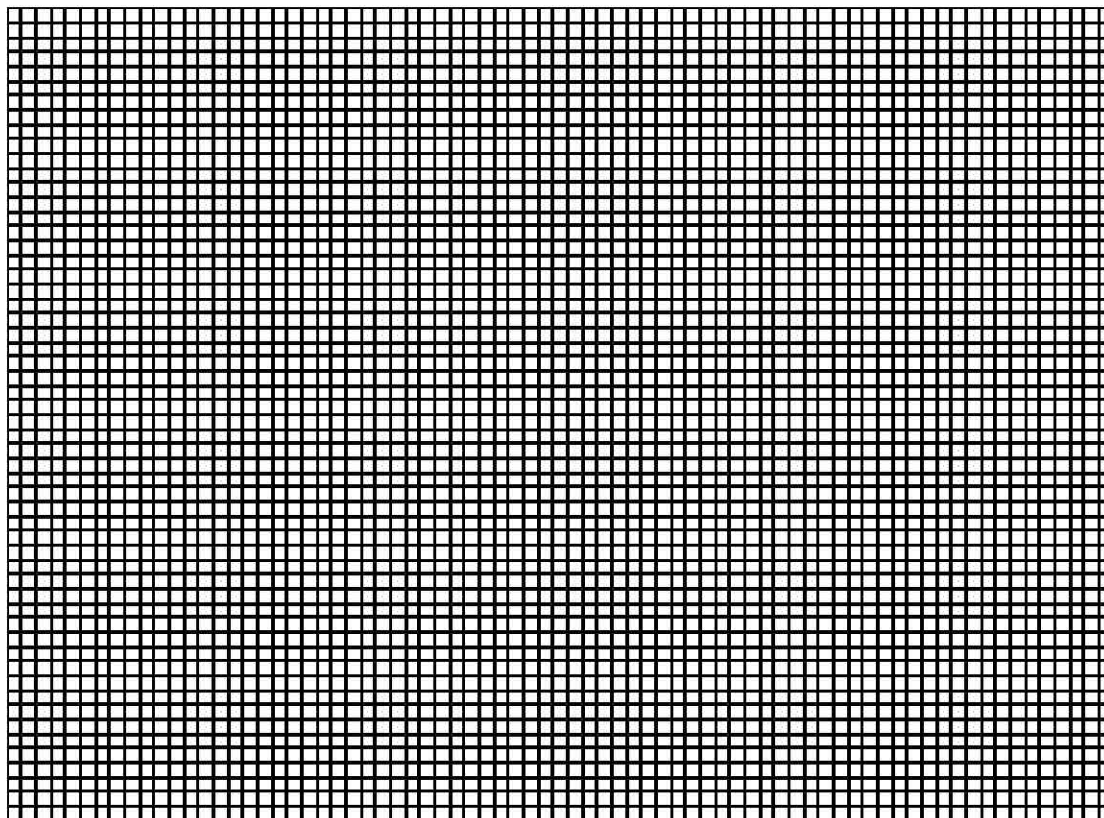
Figure 5.4. Relationship of relative net rate of photosynthesis (mean of 1/time to float) to light intensity in spinach leaf discs. Error bars represent one standard deviation above and below the means.

2.   Which is the presumed **independent variable**? _____

3.   Which is the presumed **dependent variable**? _____

4.   What relationship do you observe between Relative net rate of photosynthesis (as given by $\frac{1}{\text{time to float}}$) and light intensity?

5.   Show your calculation for the Spearman Rank Correlation test to determine if a significant correlation exists between the mean ($\frac{1}{\text{time to float}}$) and light intensity. Consult the statistics appendix. Show all your work below for full credit.

6.   What is the value of the Spearman Correlation Coefficient (R_s)? _____

7.   Fill in the following information from your test:

     H_o:

     H_a:

     Test Statistic = _____   Tabular Statistic = _____ (α = _____; n = _____)

     Decision:

8.   With reference to the collected data is the observed correlation positive or negative? _____ How do you know?

CHAPTER 6 – PROTISTS

LABORATORY SYNOPSIS

This laboratory will acquaint you with the protists and sharpen your microscopy skills. During the laboratory you will use dissecting and compound microscopes to study, in some detail, living representative species of different protistan groups so you understand better the concept of unicellular, eukaryotic organisms. This laboratory also will give you a new experience in using the compound microscope. Several of the protists you will study move rather rapidly, and keeping them in focus in the field of view requires coordination in manipulating the mechanical stage and fine focus knobs concurrently. Your microscopic study of certain protists will be supplemented with projected images of the same organisms observed with different types of microscopy.

LEARNING OBJECTIVES

Conceptual

At the end of this laboratory you should

1. be able to identify, from photomicrographs, scanning electron micrographs or living preparations, the protists studied in this lab.

2. know what a monophyletic group is and why protists are not one.

3. be able to place a protist you have studied in the appropriate major clade.

4. understand the function of a contractile vacuole.

5. know the names and basic structure of the locomotory organelles of the protists observed.

6. understand, in a general way, the feeding process of the heterotrophic organisms observed.

7. have an appreciation for the complexity of unicellular organisms.

8. know what a coenocyte is.

9. have a better understanding of the capabilities and limitations of light, scanning electron and transmission electron microscopy for studying cells.

Methodological

At the end of this laboratory you should

1. have gained proficiency in using the compound microscope to study motile specimens.

2. have sharpened your skills in preparing wet mounts for microscopic examination.

READING ASSIGNMENT

To prepare for this laboratory read:

    In Campbell and Reece, *Biology,* 8th ed. (2008):
           PROTISTS: Chapter 28.   p. 575-599

    for background on contractile vacuoles and phagocytosis, p. 107-108.

    Read through this chapter of the lab manual before coming to lab.

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                                                           Jon C. Glase

                                                           Revised June 2010
                                                           Mark A. Sarvary

INTRODUCTION

During the last four decades biologists have included a kingdom Protista in their classification system for organisms. The criteria for assigning organisms to this kingdom have changed over the years, but for the past three decades this kingdom generally has included all unicellular eukaryotes and several "simple" multicellular eukaryotes such as algae and some fungus-like organisms. The major criterion for placing a species in the kingdom Protista was the species' inability to "fit" the criteria for assignment to any other kingdom. Use of this criterion, however, was not consistent with a principal criterion used by systematists to put species into monophyletic taxonomic groups called "clades". A taxonomic group (for example, kingdom, phylum, class, etc.) is **monophyletic** if a single ancestral species gave rise to all the species in that group and to no species in any other group (Figure 6.0A). If the taxonomic grouping includes the most recent common ancestor but not all of its descendents the grouping is said to be **paraphyletic** (Figure 6.0B). One notable example of a paraphyletic group is the reptile, which should include birds as one of its descendents in order to form a monophyletic group. When the grouping of organisms is based on traits that have evolved independently it often leads to a **polyphyletic** condition. A polyphyletic group may have multiple ancestors but the most recent common ancestor is left out, as shown in the example in Figure 6.0C. In order to be a monophyletic group Ancestor 1 has to be included, thus Ancestors 2 and 3, and the descendents D and E.
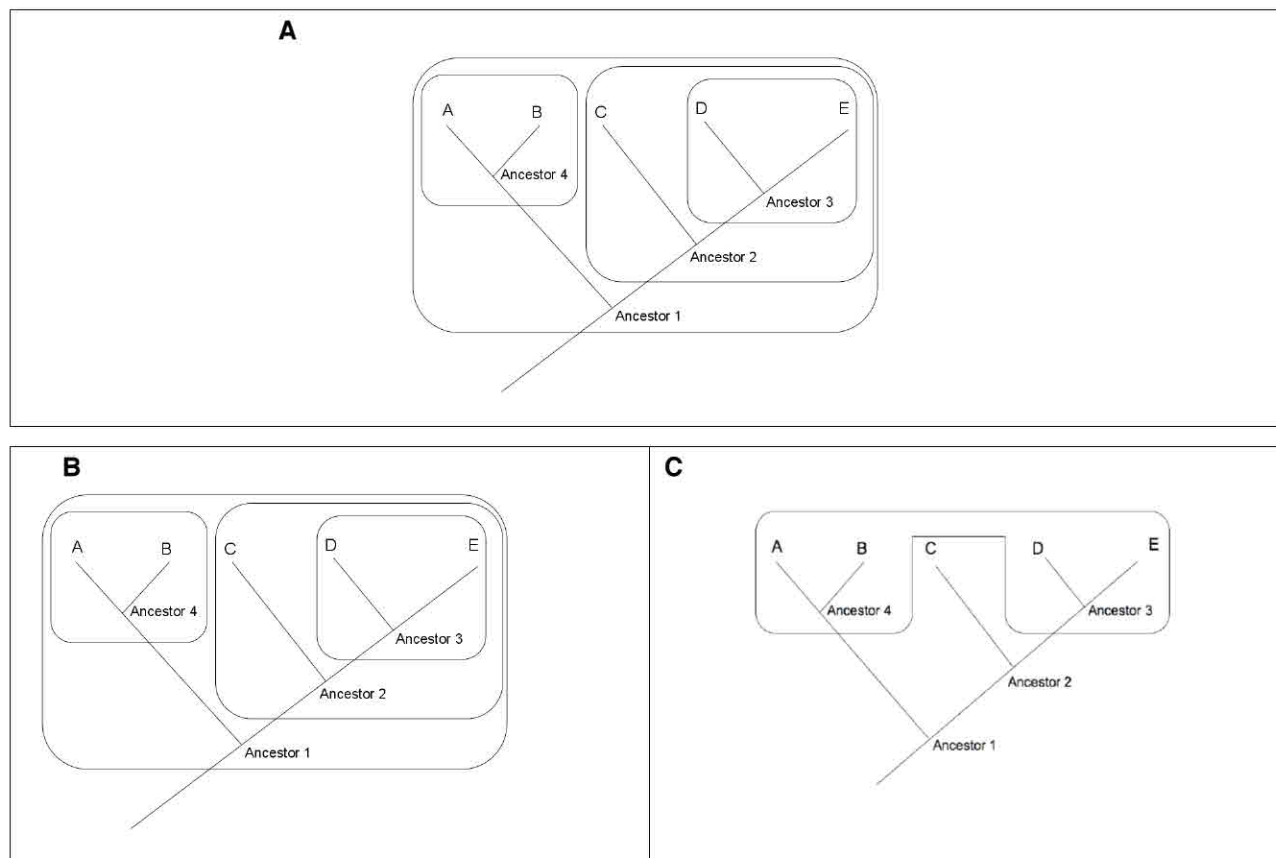
Figure 6.0. Evolutionary relationship represented through monophyletic groups. A monophyletic group, or a clade, includes the ancestor and all its descendents. In the diagram A in which A through E represent five separate taxa or classification units, such as a species, monophyly is found in four clusters, each shown in a box: Taxa A and B with Ancestor 4, Taxa D and E with Ancestor 3, Taxa C, D, and E with Ancestors 2 and 3, and Taxa A, B, C, D, and E with Ancestors 1, 2, 3, and 4. Example in B is paraphyletic. Diagram in C shows the condition of polyphyly.

Biologists have known since the inception of the kingdom Protista into biological taxonomy that it was not a monophyletic kingdom, but it was used for convenience. The grouping of protists was mainly based on traits of being eukaryotic, unicellular, plus the multicellular algae, making the protists a polyphyletic group. In the last two decades the technology of molecular biology has been applied to many different kinds of protists. The use of molecular taxonomy and ultrastructural studies has resulted in apparent evolutionary relationships among protistan groups that were not previously considered. Based on this relatively recent information, Campbell and Reece (2008) have divided the former kingdom Protista into several tentative clades, some of which may be given the ranking of kingdom in the future (See Figure 28.3, page 578 of *Biology*, by Campbell and Reece, 2008). If one were to follow this tentative grouping of protists, the current kingdom Protista will be regarded as a paraphyletic group, because it is missing Fungi, Animalia, and Plantae. Some protists have not been included in any of these groups because of the uncertainty in their evolutionary history. In this course we shall use the term protista in a general informal sense to refer to unicellular eukaryotes and closely related multicellular organisms. Algae are unicellular and multicellular protists that are photosynthetic. Protozoa are heterotrophic unicellular protists that engulf food particles. Some unicellular protists, however, are both photosynthetic and heterotrophic.

The members of many unicellular protistan species fit the general concept of a eukaryotic cell. That is, each consists of a membrane-bounded mass of cytoplasm that contains a single nucleus and several other organelles. Several unicellular protists, however, have structures that are not found in the cells of multicellular organisms. For example, some produce highly ornate and complex internal or external supporting structures of inorganic materials. Many regulate their internal water and ion content by using an apparatus, composed of membrane sacs and microtubules, called a "contractile vacuole" or a "water expulsion vacuole." Minute thread-like ejectile structures are used by some predatory protists to capture prey.

Many different kinds of unicellular protists use slender appendages called flagella or cilia for locomotion. The flagellum of eukaryotic cells and the cilium are basically the same structurally, except the flagellum is longer.

Many unicellular protists have more than one nucleus (some have more than a hundred nuclei!), but they are regarded as unicellular because each consists of a single body of cytoplasm surrounded by a plasma membrane. In most of the groups whose members are multinucleate cells, all the nuclei in a cell apparently are the same. However, the ciliates are distinguished from other protists by having two types of nuclei in a "cell": (1) a large macronucleus and (2) a smaller micronucleus. The macronucleus is polyploid; that is, it contains many sets of chromosomes.

The ciliates are the most complex unicellular protists. They use cilia, organized in a variety of forms, for locomotion and feeding. Food is taken in through a distinct and fixed part of the cell called a cytostome (cell mouth) and indigestible wastes are eliminated from the cell at a specialized region.

Biologists have difficulty interpreting the organizational level of certain protists, especially the ciliates. They may be considered as unicellular organisms because each is a cytoplasmic unit surrounded by a membrane, but their complexity and diversity of structures far exceed that of cells in multicellular organisms. Consequently, the more complex unicellular protists are interpreted by some biologists to be "acellular" organisms. The term acellular implies that these organisms are the equivalents of simple multicellular organisms that are not partitioned into several cells.

In this laboratory exercise you will study selected organisms from the algae, protozoans and fungus-like protists. Specimens were selected to show variation in structure and locomotory and feeding methods. All of the protists you will observe are free-living, except the termite endosymbionts, and all of the free-living aquatic protists are freshwater organisms. Listed below are the tentative major clades, in uppercase letters, as named by Campbell and Reece (2008) and smaller clades represented by the specimens you will observe.

RADIOLARIA (ACTINOPODA)
    Heliozoans

PARABASALIDA
    Polymastigotes and Hypermastigotes

ALVEOLATA
    Dinoflagellates
    Ciliates

STRAMENOPILA
    Diatoms

EUGLENOZOA
    Euglenids

CHLOROPHYTA
    Chlorophytes

AMOEBOZOA
    Gymnamoebas
    Plasmodial Slime Molds

GENERAL PROCEDURES AND RELATED INFORMATION

The following text is designed to be a guide for your preparation and study of selected protists. A table is provided at the end of this chapter to help you summarize the important characteristics of the protists you observe. You may fill in the table as you study the organisms or complete it later as a review. To sharpen your observational skills and for future reference, it is a good idea to sketch what <u>you actually see</u> when observing microscopic specimens.

Cultures of protists are available in tubes or jars, each with a labeled pipette for dispensing a sample. Some culture jars are on dissecting microscopes which you should use to help you find the organisms you wish to remove.

❏ Each person needs a compound microscope and a dissecting microscope. Each group needs one pair of polarizing filters. Each member can prepare a different specimen for observation. Be sure each member in a group studies every specimen. If you have a particularly good preparation, consult your lab instructor; s/he may wish to use it for a laboratory display.

❏ Review steps 1-7 on page 37 when you set up your compound microscope. Be sure to adjust the iris diaphragm to give you the best resolution. Appropriate light intensity is extremely important when viewing protists. Many of their fine details cannot be seen if the light intensity is too high.

❑   Please use the labeled pipette only in the protistan culture for which it is labeled. Prepare mounts, as described for each species, on clean microscope slides. Before applying a coverslip to your preparation, scan it with a **dissecting** microscope to see if the desired organisms are present. If you cannot find the organism you are looking for, ask your instructor for assistance.

❑   When you are certain you have the appropriate organism on your microscope slide, cover the sample with a coverslip in the following way. Place and hold one edge of the coverslip on the slide near the sample. Slowly lower the coverslip onto the sample. This procedure minimizes the trapping of air bubbles in the covered liquid.

❑   Begin your observations of each organism using the 4X (scanning) objective on your compound microscope. This objective gives you the largest field of view and enables you to find the moving organisms more easily. After locating an organism, use greater magnification to study it.

Initially you may have difficulty keeping a moving organism in the field of view. To view a motile organism you must continually adjust the mechanical stage and the fine focus knob to bring the organism back into view and into focus.

One, two or all of the following special procedures may be specified in the procedure to prepare certain protists for microscopic viewing.

Use of Methyl Cellulose Solution

A solution of **methyl cellulose** (trade name, Methocel), which has a high viscosity, can be used to reduce the speed of rapidly moving organisms. When the use of methyl cellulose is specified in a procedure, it should be used as follows:

❑   Using a pipette, dispense a thin ring of methyl cellulose solution on the middle of a clean microscope slide. The diameter of the ring should be 1/2 to 2/3 the width of a coverslip. Remove air bubbles from the liquid by drawing them into the pipette. Dispense one or two drops of the desired protistan culture into the center of the ring. After you are certain the desired organisms are on the slide, cover the liquid with a coverslip. Keep the slide level while transferring it to the microscope's stage.

As the organisms move outward from the center into the more viscous liquid, their rate of movement is decreased.

Use of Coverslip Spacers

Some protists require additional space between the slide and the coverslip to prevent them from being squashed by the coverslip. When the use of coverslip spacers is mentioned in the procedure, do the following:

❑ Place two coverslips about 1.5 cm (5/8 in) apart, with the liquid between them, on the slide. Place a third coverslip directly over the liquid so it is supported by the two coverslips. See Figure 6.1.

Coverslip over liquid with specimens
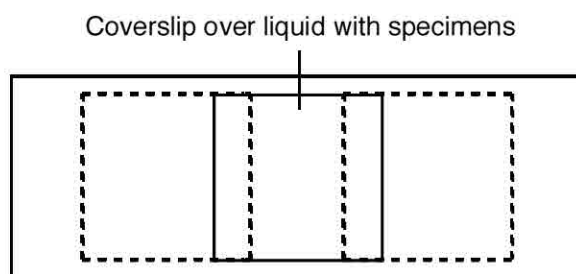
Figure 6.1. Top view of a microscope slide showing positions of coverslip spacers (broken lines) used to raise the coverslip over the liquid containing the specimens.

Use of Dark Field Microscopy

Two microscopes set up for dark field illumination will be available for your use. After studying an organism in bright field illumination on your compound microscope, you might wish to observe it in dark field illumination. Some of the protists have numerous structures which refract and diffract light, and thus produce remarkable images in dark field illumination.

Tentative clade RADIOLARIA

HELIOZOANS: *Actinosphaerium* sp.

❑ Use the dissecting microscope to scan the contents of the *Actinosphaerium* culture jar. Look for stationary spheres among the debris, but not on the bottom of the jar. While viewing through the microscope, use the *Actinosphaerium* pipette to remove one or more of the spheres and transfer them to a clean microscope slide. (DO NOT USE METHYL CELLULOSE WITH *Actinosphaerium*).

❑ Use your <u>dissecting</u> microscope to confirm that *Actinosphaerium* is on the slide. After you find the organism, prepare the slide with coverslip spacers and again use the <u>dissecting</u> microscope to find the location of the organism(s) on the slide.

❑ Observe the preparation with a compound microscope at low magnification to find *Actinosphaerium*, which looks like a sphere with numerous "spines" radiating from it.

Actinosphaerium does not have locomotory structures. It is a planktonic drifter, which is carried by the water current. Its numerous projections give it a large surface area in contact with the water which provides buoyancy.

❑ Observe *Actinosphaerium* with high magnification. Focus up and down through the protoplasmic sphere.

The sphere of the organism consists of an outer layer of highly vacuolated ectoplasm and an inner sphere of more dense endoplasm. The nucleus, which is not distinguishable without staining, is in the endoplasm. You may see small organisms in food vacuoles within the endoplasm. See Figure 6.8.

The slender extensions radiating from the sphere are called **axopodia**. Unlike the pseudopodia of amoebas, axopodia do not change their shape. The rigidity of an axopodium is maintained by the presence of an axial rod composed of a bundle of microtubules. The axial rod is covered by a thin layer of cytoplasm which is contained by the cell membrane. Various microorganisms that stick to the surface of the axopodia are engulfed (phagocytized) by the thin layer of cytoplasm. Captured prey are then transported to the central sphere by cytoplasmic streaming.
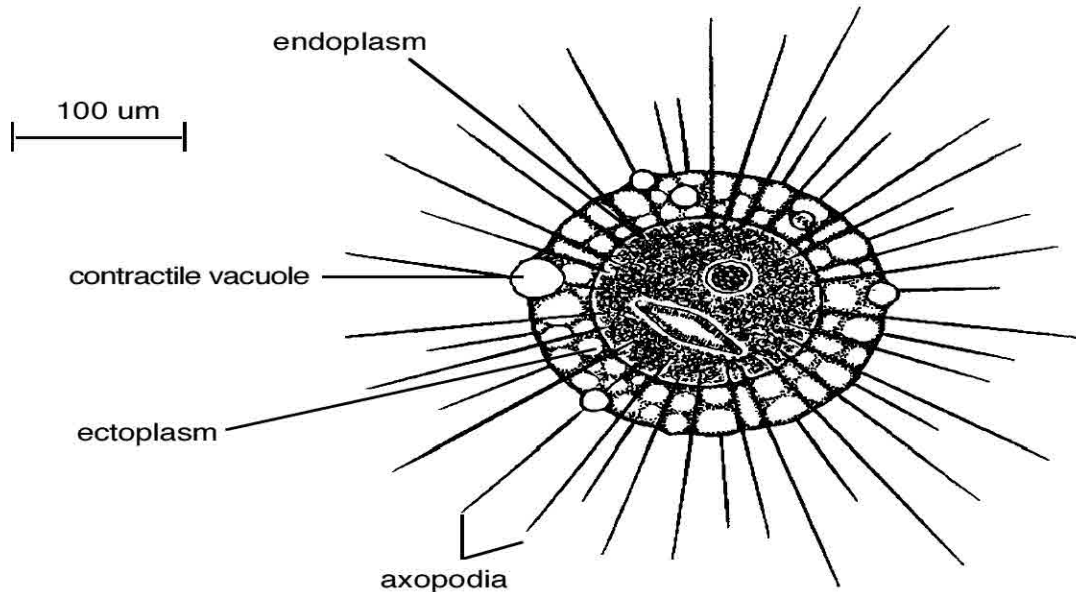
Figure 6.2. *Actinosphaerium sp.;* adapted from Jahn and Jahn (1949).

Tentative clade PARABASALIDA

Parabasalids are flagellated protists that are parasitic or symbiotic in anaerobic environments of host organisms. Their mitochondria lack mechanisms for electron transport and enzymes for the citric acid cycle.

***Trichonympha* sp.**

Protozoans (animal-like protists) with flagella such as this group, *Trichonympha* spp., and a few other flagellated protistan species live in a symbiotic relationship with termites. These protists occupy the termite's gut where they digest wood particles eaten by the insect. Termites lack the enzymes necessary to digest the wood they have ingested and depend entirely on *Trichonympha* to make the nutrients in wood available to them. *Trichonympha* cannot survive without its termite host; it is so well adapted to the environment of the termite's gut that it cannot live long or reproduce when removed. Since *Trichonympha* can survive only in the culture medium of the termite's gut, that is where you must obtain it.

❑ Using insect forceps, obtain a mature termite from the moist paper or wood in the culture jar. Hold the insect on a clean microscope slide with insect forceps and carefully squeeze its abdomen with another insect forceps until it releases feces, which contain the protists. Immediately add one drop of 0.6% NaCl solution to the preparation. Do not add water to the preparation; it causes the

zooflagellates to swell and burst. Return the termite to its container. Use coverslip spacers on this preparation (see Fig. 6.1). Observe the preparation with low magnification. These organisms are rather transparent; consequently you may have to reduce the light intensity to see them.

Trichonympha is a bell–or egg–shaped organism with hundreds of shimmering flagella. See Figure 6.3. These organisms are very densely packed in the termite's gut and may account for as much as one-third of a termite's weight. Your termite may have other species of protists in addition to *Trichonympha.*

❑   After obtaining a good view of the *Trichonympha*, use high magnification to study them in more detail.

The numerous flagella may mask internal structures, but careful focusing and adjustment of light intensity might allow you to see some internal details. The nucleus lies approximately in the middle of the organism. The broader (posterior) part of the organism is likely to contain small wood particles in vacuoles. *Trichonympha's* posterior region is sticky and amoeboid. Wood particles cling to the organism and are taken in by phagocytosis, amoeba–style. Following digestion of the wood, some of the soluble digestion products are secreted by the protists to nourish the termite.

The function of numerous flagella on these organisms is not understood. Within the termite's gut the protozoans live in high density and in a highly viscous medium, where movement by flagellar action seems unnecessary and perhaps impossible.

❑   Observe *Trichonympha* using dark field microscopy, and under bright field using polarizers.
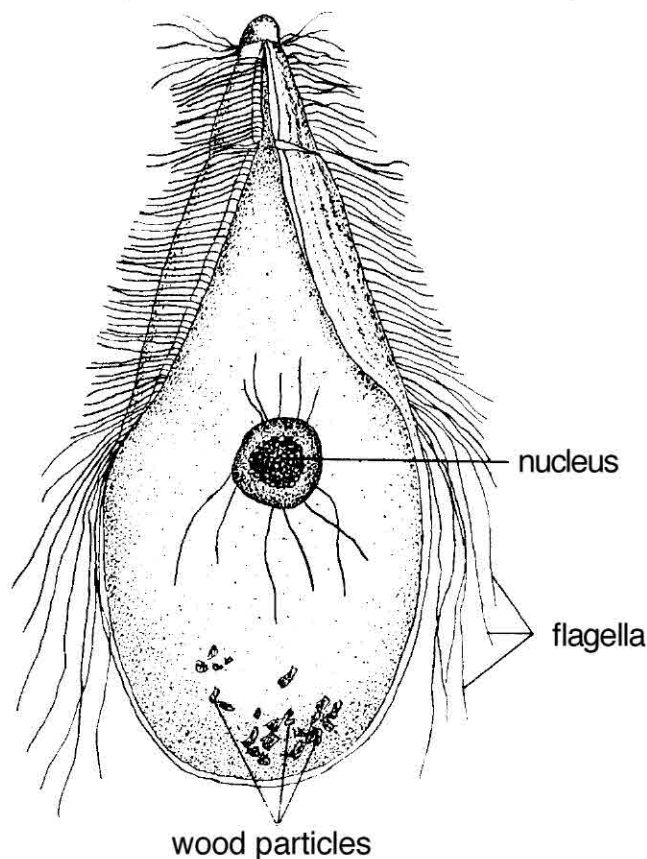
Figure 6.3 Cutaway view of *Trichonympha* to show internal structures. The entire surface of the organism, except for the posterior end, is covered with flagella.

Tentative clade ALVEOLATA

CILIOPHORA (CILIATES)

The ciliates are generally regarded as the most complex protists. They share the following distinguishing characteristics: (1) possession of cilia as locomotory organelles, (2) possession of two kinds of nuclei, (3) a distinct form of asexual reproduction resulting from characteristic 2, and (4) a unique type of sexual reproduction. You will observe the first two characteristics in this study.

The three species representing the Ciliophora were selected to show the various ways the cilium is used by different members of this phylum. *Paramecium* uses only the basic cilium as a locomotory and feeding organelle, while *Stentor* and *Euplotes* have organelles which are specialized bundles or rows of cilia.

❑   Each member of a group should prepare one of the three ciliates for observation as describe below. The three specimens may be prepared at the same time, but each person should observe *Paramecium* before the other two.

Studying *Paramecium* first will enable you to be already familiar with the typical structures of ciliates when observing *Stentor* and *Euplotes*, and you can better appreciate their structural complexity. Furthermore, the time interval between preparation and observation of *Stentor* and *Euplotes* samples allows the organisms to slow down so they can be observed more easily.

Paramecium caudatum

❑   Prepare a sample of *P. caudatum* with methyl cellulose solution on a clean microscope slide. You are most likely to obtain organisms in the debris on the bottom of the culture jar. Use low magnification to find the organisms and to observe its overall form and movement. Notice a distinct groove, called the **oral groove**, running diagonally along half the organism's length (see Figure 6.4).

Paramecium caudatum is one of the larger species of this genus. Like all *Paramecium* species it is more or less slipper–shaped. This streamlined shape is a general adaptation for rather rapid locomotion through an aquatic environment. *Paramecium* has distinct anterior and posterior ends, based on the usual direction of movement and position of the feeding apparatus. The organism rotates about its longitudinal axis as it moves. Is this rotation clockwise or counterclockwise?

❑   Find an organism which is relatively inactive and study it using high magnification. Refer to Figure 6.4 while attempting to see the structures mentioned.
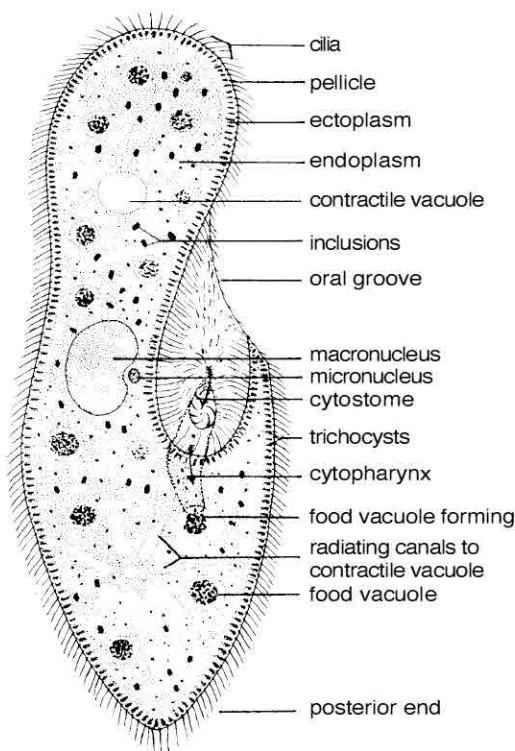
Figure 6.4. Diagram of *Paramecium caudatum;* modified from Wodsedalek and Lytle (1963).

The entire surface of the organism has **cilia** projecting through the **pellicle**, or outer covering. You can see beating cilia by carefully focusing and adjusting the light intensity. The pellicle of *Paramecium* is a complex structure consisting of an outer membrane, which is the organism's outer surface, and an inner membrane which forms a lattice to support the cilia and **trichocysts**. Basal bodies of cilia and trichocysts are embedded in the thin transparent ectoplasm, the outer layer of cytoplasm. **Trichocysts** are minute harpoons in a contracted form in the ectoplasm. If exposed to the proper stimulus, trichocysts discharge to form threadlike filaments with pointed tips that protrude from the organism.

Paramecium uses the special cilia in the **oral groove** for collecting the bacteria, yeast, and particulate organic material constituting its main diet. It has been estimated that *P. caudatum* requires in excess of 1500 individuals of the bacterium *Bacillus subtilus* per hour for normal growth. Because of this, paramecia spend most of their time feeding. As the organism moves about, food particles become trapped in the oral cilia and are swept into a pouch–like structure, called the **cytopharynx** (look carefully, you can see it), located at the back of the oral groove. The opening to the cytopharynx is frequently called the **cytostome** (cell mouth). Invagination at the base of the cytopharynx forms food vacuoles, which are moved about within the organism by cytoplasmic streaming. Digestive enzymes are added to these food vacuoles by their fusion with lysosomes and any undigested matter is discharged at a special point on the posterior surface of the cell called the **cytopyge** or **anal pore**.

Paramecium has two **contractile vacuoles**, one at each end, to remove surplus water. Each vacuole is surrounded by **radiating canals** which collect excess water from cytoplasm and slowly fill the vacuole. When filled, the vacuole fuses with the pellicle and contracts, expelling its contents to the outside. If you are patient, you might see a vacuole contract.

P. caudatum possesses two types of nuclei: a large **macronucleus** which regulates the day–to–day metabolism of the organism, and a **micronucleus** which is involved exclusively in sexual reproduction. The macronucleus is located near the center of the cell with the micronucleus beside it.

❑ Observe fixed, stained whole mounts of *Paramecium caudatum* on a prepared microscope slide to see structures you could not observe on/in the live specimens.

❑ Using the fixed mounts, measure the length of *P. caudatum* by using the mechanical stage scales.

initial reading: _____

final reading: _____

length of *P. caudatum*: _____

Stentor coeruleus

❑ Using a dissecting microscope with a white stage plate, scan the *Stentor* culture jar until you find some of the blue organisms, which may be swimming freely or attached to debris. While looking in the microscope, use the *Stentor* pipette to carefully remove a few organisms. Attempt to get one or two attached to debris. Transfer two drops of your sample to a clean microscope slide, and observe it with the dissecting microscope to see if you dispensed *Stentor* onto the slide. When you are certain *Stentor* is on the slide, use coverslip spacers to prepare it for observation. *Stentor* should not need methyl cellulose to slow it, especially if it is attached to debris.

❑ Find, and initially study, one or more individuals using low magnification on your compound microscope.

Organisms may be free–swimming or attached to debris particles. Free–swimming organisms are pear shaped while attached organisms are trumpet shaped.
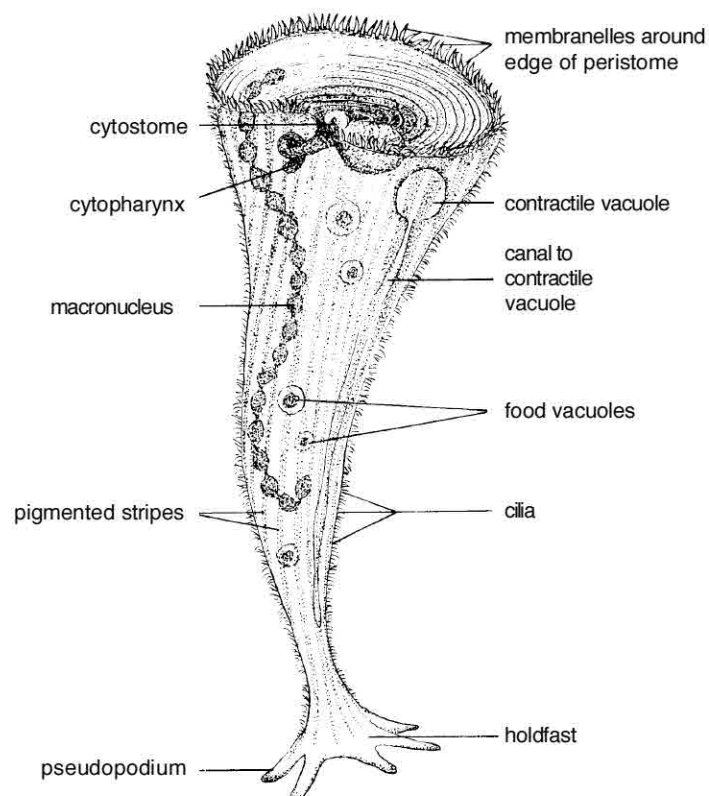
Figure 6.5. *Stentor coeruleus* displaying the trumpet shape of the attached organism.

❑   Refer to Figure 6.5 as you study *Stentor*.

Stentor coeruleus has longitudinal, alternating clear and blue stripes which converge at its posterior end. The specific name describes the cerulean blue color of this species. A row of **cilia** runs the length of each clear body stripe. Locomotion is accomplished mainly by ciliar action, but beating **membranelles** on the anterior end assist.

A **membranelle** is a cluster of cilia, two or three rows thick and approximately 20 cilia long. All the cilia adhere to each other and beat together as a unit. The basal bodies of these clustered cilia are bound together in a membranous bundle. The basal body bundle of each membranelle is connected through a root-like system of fibrils to adjacent membranelles.

Whether *Stentor* is free or attached you can see the ring of beating membranelles around its anterior end. When the organism is attached to a substrate, its body is extended and the anterior end expands to reveal a funnel–shaped feeding apparatus consisting of a flared **peristome** (or **buccal cavity**), which tapers to the **cytostome** (cell mouth), the opening to the pouch–like **cytopharynx.** The membranelles around the ridge of the peristome beat in a smooth rhythm, one after the other in a clockwise direction, creating a vortex that draws particles into the peristome. The beating of cilia in the peristome is coordinated to spin the particles toward the cytostome. Accepted food particles enter the cytopharynx; rejected ones are removed from the peristome apparently by a reversal of the ciliary beat. Formation of food vacuoles and elimination of undigested material is like that in *Paramecium*.

Stentor attaches itself to objects with a **holdfast** composed of **pseudopodia** formed by amoeboid movement. Extension and contraction of the body is accomplished by the activity of **myonemes**, slender contractile threads which run the length of the organism between the blue pigmented stripes.

Stentor has one **macronucleus** consisting of several nodes and appearing like a string of beads. Several **micronuclei** are present close beside the macronucleus. A single **contractile vacuole** is located in the anterior region. The contractile vacuole has a single canal along the length of the organism.

Euplotes sp.

❑   Observe the *Euplotes* culture with a dissecting microscope using a black stage plate. When you have located the organisms (look in the debris on the jar's bottom) carefully remove a few with the pipette and prepare a sample with methyl cellulose solution on a clean microscope slide. Use low magnification on the compound microscope and persistence to find the organisms.
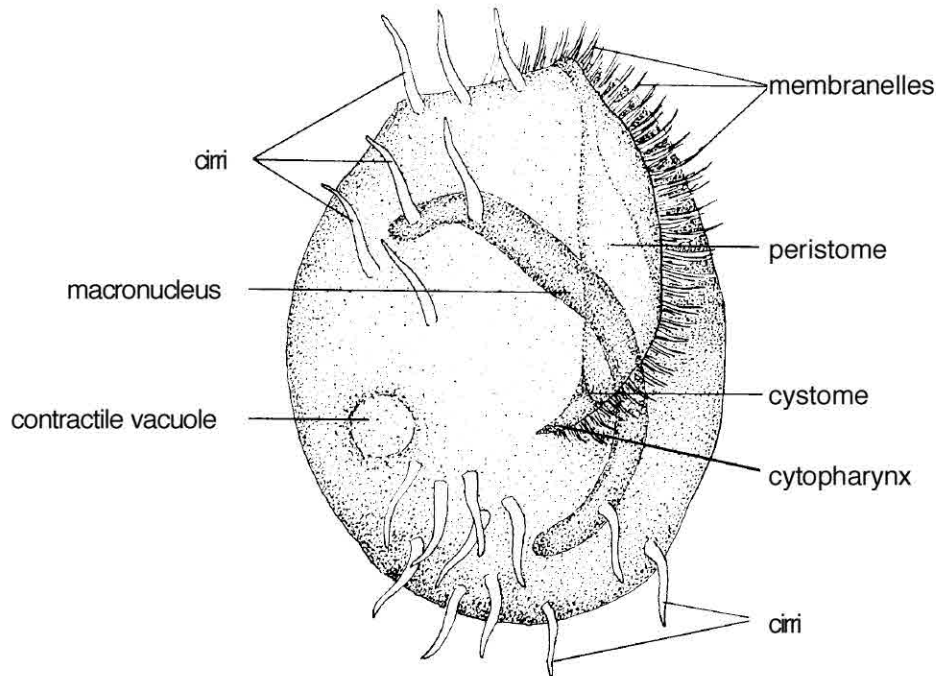
Figure 6.6. *Euplotes* sp. The undulating membrane is not shown in this diagram.

The most striking feature of *Euplotes* is the presence of legs used for swimming or walking on objects. The legs are **cirri** (singular, **cirrus**). Each cirrus is a circular bundle of cilia tapering to a point at the distal end. The cilia adhere to each other and move as a unit. Unlike *Paramecium* and *Stentor*, *Euplotes* has very few individual cilia. When an organism swims it occasionally rotates so you can see its distinct convex dorsal and flat ventral surfaces.

❑   Find an organism which is relatively still and observe it with high magnification.

Within the relatively transparent body, you can see a C–shaped **macronucleus**, a single **contractile vacuole** and perhaps a few **food vacuoles** containing bacteria or small protists. Refer to Figure 6.6.

Much of the ventral surface is the **peristome** (or **buccal cavity**), a scoop–shaped depression, which leads to the **cytostome** (cell mouth) and the **cytopharynx**. A row of membranelles (see description under *Stentor*) begins on the dorsal surface and runs clockwise down onto the ventral surface along the edge of the peristome. Within the peristome is a row of membranelles and opposite it, a small **undulating membrane**, which is not a true cellular membrane but a single row of cilia that move together as an undulating sheet. The membranelles and undulating membrane function collectively to bring food particles into the peristome and direct them toward the cytostome. As in *Paramecium* and *Stentor*, food vacuoles are formed at the internal end of the cytopharynx, and undigested material is removed via an anal pore.

All the organelles composed of cilia– cirri, membranelles and the undulating membrane– can function concurrently in a coordinated way. Furthermore, they can move separately; membranelles can beat while cirri are motionless, and one or more cirri can move independently. Microscopists have found within the body of *Euplotes* a network of interconnecting fibrils that appear to link cirri with each other and with membranelles. (Also recall that the membranelles of *Stentor* and the cilia of *Paramecium* appear to be connected by a fibrillar system.) These observations and others have led some researchers to hypothesize that ciliates possess subcellular neuromotor apparatuses which regulate the activities of their locomotory and feeding organelles.

***Vorticella* sp.**

❑ Observe the prepared display of *Vorticella*. Watch it long enough to see the organism contract.

Vorticella, a ciliate, looks like a bell on a long stalk when it is feeding. It is found attached to submerged objects including aquatic plants and animals. Three rings of cilia in single file are around the edge of the flared **peristome**. Sequential beating of the cilia create a whirling motion in the liquid in the peristome, which draws water and food particles into a **peristomal funnel** connected to the **cytostome** (cell mouth). The body and stalk do not have cilia. The most remarkable feature of *Vorticella* is its contractile stalk. Rapid contraction of the stalk is accomplished by the activity of myonemes, contractile filaments that run the length of the stalk. Notice how the stalk coils when it contracts. Does the stalk extend itself as rapidly as it contracts?

❑ Make sketches of *Vorticella* in its extended and contracted forms.

ALGAL PROTISTS

Except for cyanobacteria (blue-green algae), all organisms generically called algae are included in the protists. Some biologists used to place the green, brown, and red algae in the plant kingdom, but now evidence shows that they are more closely related to protists. All photosynthetic protists have chlorophyll *a*, as do the cyanobacteria and plants. The accessory pigments vary among the algal protists and are used—along with cell-wall chemistry, stored food products, and chloroplast structure—as classification characteristics.

Tentative clade ALVEOLATA: DINOFLAGELLATA: *Peridinium sp.*

❑ Thoroughly mix the *Peridinium* culture with the dropper in the culture jar, and then prepare, on a clean microscope slide, a sample of the culture with methyl cellulose solution. Use the lowest magnification on the compound microscope to find the organisms; then study them at higher magnification.

Peridinium sp. is photosynthetic and, therefore, is an algal protist. Peridinin, a brown pigment, masks the green chlorophyll, making the chloroplasts appear yellow-brown. *Peridinium* and related genera belong to a group called dinoflagellates because of their type of locomotion. The term dino means whirling or spinning, and as you can see, these organisms whirl as they are propelled by their flagella.

Each organism is armored with a layer of discrete, sculptured, cellulose plates jointed in a definite pattern. See Figure 6.7. The details of the armor are seen better with high magnification of stationary organisms. Unlike most walled cells, which have their walls outside the cell membrane, *Peridinium* and other armored dinoflagellates have their cellulose walls inward of the cell membrane. Some pieces of armor without the organism also may be seen.
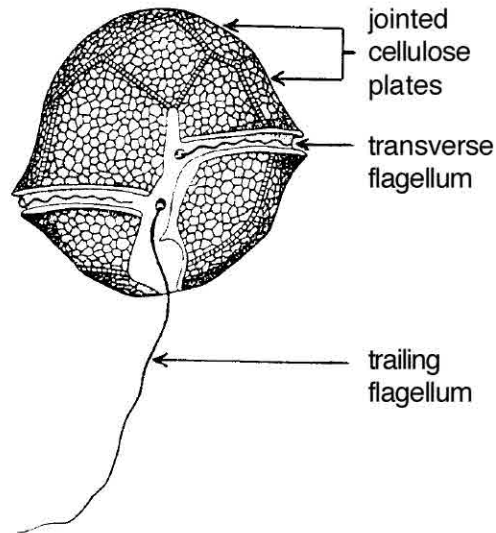
Figure 6.7 *Peridinium* sp. (a dinoflagellate).

A dinoflagellate has two dissimilar flagella emerging close together. A broad ribbon-like flagellum, encircling the cell in a transverse groove, undulates to make the organism spin. The other flagellum, which is whip-like, lies in a longitudinal groove and trails behind this organism. Undulations of this trailing flagellum provide forward motion and contribute somewhat to the rotation of the organism. Using high magnification and relatively low light intensity, you might be able to see the flagella of *Peridinium*.

The cellulose cell wall of *Peridinium* does not allow you to see its intracellular structures easily.

Tentative clade STRAMENOPILA: BACILLARIOPHYTA (Diatoms)

Diatoms, of the Bacillariophyta, are unicellular photosynthetic protists that are abundant in freshwater and marine habitats. As the largest component of marine plankton, diatoms are the major photosynthetic producers in marine communities. They are noted for their unique and beautiful glasslike shells. Huge fossilized deposits of diatom shells are mined as diatomaceous earth, which has numerous commercial uses.

❑   Look at the drawings and photographs of diatoms posted in the laboratory. Note how their shells may have a variety of shapes and ornamentations. These physical characteristics form the basis for their classification.

❑   Look for live diatoms in the pond water and hay infusion cultures later in this lab.

Tentative clade EUGLENOZOA: EUGLENOPHYTA: *Euglena gracilis* and *Peranema* sp.

❑   Prepare wet mounts of *Euglena gracilis* and *Peranema* sp. with methyl cellulose solution on a microscope slides. Use low magnification to find the organisms and then change to high magnification to observe them.
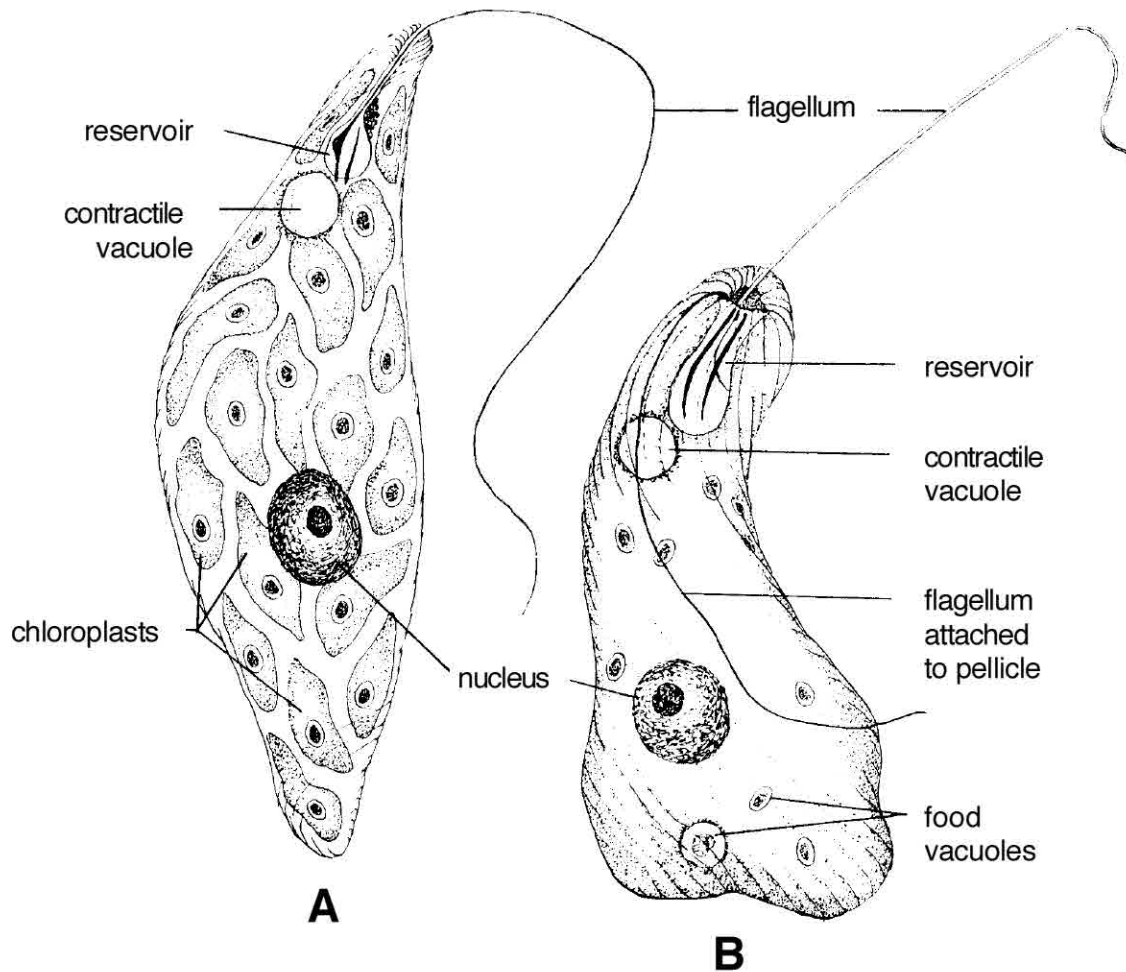
Figure 6.8. Representatives of Euglenozoa. A. *Euglena gracilis*, B. *Peranema* sp.

Euglena gracilis is photosynthetic, containing several chloroplasts. See Figure 6.8 A. It also can obtain organic nutrients by absorption. The organism is covered by a **pellicle** consisting of the cell membrane on the outside and a layer of overlapping strips of proteinaceous material immediately inside the membrane. *E. gracilis* has two flagella originating in the base of an anterior invagination called a **reservoir**. One flagellum does not extend outside the reservoir. With careful focusing and adjustment of light intensity, you can see the reservoir and the one protruding flagellum. Two relatively transparent bodies can be seen within the organism–the **nucleus**, which is about midway along the organism's length, and a **contractile vacuole**, which is adjacent to the reservoir and empties into it.

❑ Look for an orange-red spot near the anterior end (the end with the flagellum) of each individual.

The orange-red spot is a pigment granule called the eyespot. It is involved in light detection and subsequent phototactic responses of the organism.

Peranema sp. is structurally similar to *Euglena gracilis*, except it has no chloroplast. See Figure 6.9.B. It ingests its food, which may be other protists including *Euglena*. Instead of chloroplasts it may have several **food vacuoles**. *Peranema* has two flagella; both protrude from the reservoir. One flagellum is attached to the pellicle along the length of the organism and is not easily seen. The **locomotory flagellum** is quite obvious. Notice that most of the time the locomotory flagellum is extended rigidly with only its distal portion undulating.

CLADE CHLOROPHYTA (Green Algae): *Volvox*

The chloroplasts of green algae resemble those of plants in structure and pigment composition, and botanists believe that plants evolved from chlorophytes. Most species live in freshwater. Various unicellular forms may be planktonic, soil inhabitants, or symbionts in invertebrates or in fungi (forming associations known as lichens). Colonial species may form flagellated balls of cells or filaments. Some large multicellular forms resemble plants, but lack true roots, stems, and leaves. In this lab you will study *Volvox*, a spherical colonial species.

The life cycles of most green algae include sexual and asexual stages. Some multicellular forms have an alternation of generations. Sexual reproduction probably originated through the fusion of morphologically identical haploid cells, a condition called isogamy. The life cycle of the unicellular green alga, *Chlamydomonas*, illustrated on p. 592 of Campbell and Reece (2008), illustrates isogamy.

The green alga, *Volvox* illustrates heterogamy, sexual reproduction that involves gametes that are clearly different. The difference can be of size or motility; the most extreme form of heterogamy, in which the female gametes are large, nonmotile eggs and the male gametes are small, motile sperm, is called oogamy. *Volvox* is also an example of the organization of cells into colonies with associations between the cells and consequent division of labor; cells become specialized for particular tasks.

❑   Make a wet mount of *Volvox* from the live culture. *Volvox* colonies vary in size from several hundred to as many as 40,000 cells arranged in the wall of a hollow sphere and interconnected by cytoplasmic strands.

❑   Asexual reproduction occurs through the formation of daughter colonies which develop mitotically from single vegetative cells and are eventually freed into the interior of the parent sphere. Look for some living colonies with daughter colonies in the interior of the parent sphere.

When the parent colony dies and breaks apart, the daughter colonies are freed. You may be able to find parent colonies in various stages of degeneration, with daughter colonies inside.

Sexual reproduction is oogamous; eggs and sperm are formed (Figure 6.9). *Volvox* shows a marked division of labor; only a few of the cells are active in gamete production, while the rest are permanently vegetative.
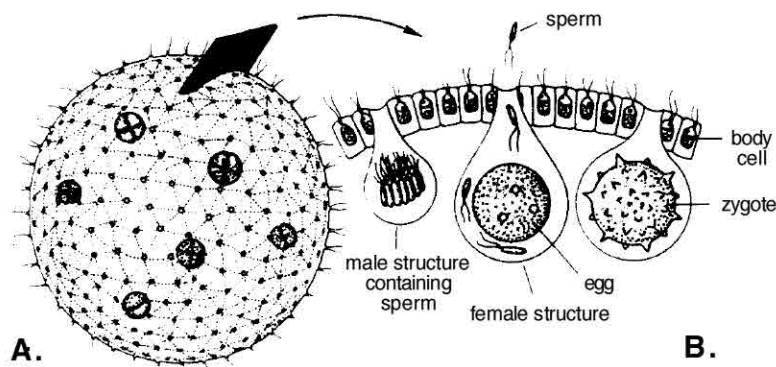
Figure 6.9. ***Volvox.*** **(A) Living vegetative cells with many daughter colonies in the interior of the parent colony. (B) Section through the surface of a colony, showing male and female reproductive structures. Sperm released by the male structures enter the female structure and fertilize the egg. After a period of inactivity, the zygote divides meiotically and then the resulting haploid cells divide mitotically, producing a new daughter colony, which is eventually released. (A. Courtesy Carolina Biological Supply Company. B. Modified from H.J. Fuller and O. Tippo, College Biology, 1949).**

Tentative clade AMOEBOZOA

Gymnamoebas: *Amoeba proteus*

❑ Using the dissecting microscope with the culture jar, scan the bottom of the *Amoeba* culture jar until you find one or more irregularly shaped granular bodies whose shape is changing slowly. While viewing through the microscope, use the *Amoeba* pipette to remove a few of these organisms from the culture jar. Dispense two or three drops of the culture onto a clean microscope slide (DO NOT USE METHYL CELLULOSE WITH *Amoeba*).

❑ Use your dissecting microscope to confirm that *Amoeba* is on the slide. After you find *Amoeba*, prepare the slide with coverslip spacers (see above).

❑ Observe the preparation with your compound microscope at low magnification. Again, look for an irregularly shaped granular body whose shape is slowly changing by the formation of one or more extensions. When you have found an *Amoeba*, carefully observe its manner of movement.
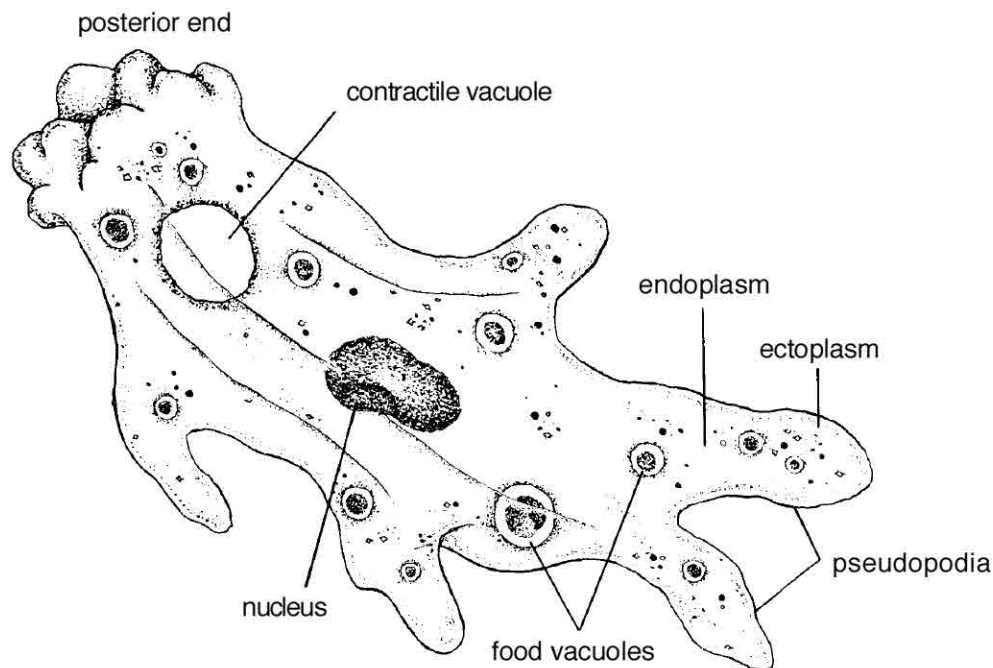
Figure 6.10. *Amoeba proteus.*

The cytoplasmic extensions formed by amoeboid cells are called **pseudopodia** (false feet), by which the cell moves and captures food. An amoeba has a definite anterior and posterior end. The latter is a wrinkled region that does not form pseudopods and is pulled along as the organism moves. See Figure 6.10.

The outer layer of *A. proteus* is the **plasma-** or **cell membrane**. Immediately beneath it is a thin transparent layer of cytoplasm called ectoplasm, which is most clearly seen at the tip of a pseudopodium where it forms a transparent cap. The bulk of the cytoplasm is endoplasm which contains all the visible organelles. The **nucleus**, one of the largest cytoplasmic structures, is densely granular in appearance. Numerous **food vacuoles** and smaller granular materials can be seen moving in the endoplasm. One of the largest organelles is the **contractile vacuole**, a transparent sphere. Observe the organism carefully, you may be able to see the contractile vacuole contract.

Contractile vacuoles are found in members of many protistan groups. The organelle collects and eliminates excess water, often with dissolved salts and metabolic wastes. When the vacuole is full, its membrane fuses to the cell membrane and it then contracts, forcing its contents outside the cell. Mainly freshwater protists, which passively absorb water from their environment, have contractile vacuoles, but some marine ciliates also possess them.

❑   Again consider the movement of *A. proteus*. Notice that endoplasm flows forward from the posterior region of the cell into the extending pseudopodium and then toward the sides at the tip of the pseudopodium.

This amoeboid movement involves changes in the viscosity of endoplasm. Peripheral endoplasm, except at the tip of a pseudopodium, is a stationary, semisolid substance. In the posterior region semisolid endoplasm is converted to a fluid endoplasm that flows into the pseudopodium. At the pseudopodium's tip the fluid endoplasm flows to the sides where it is converted to the semisolid form. The peripheral semisolid endoplasm provides rigidity to the sides of the pseudopodium and channels the flow of fluid endoplasm to extend the structure. Whether the organism pulls or pushes itself by amoeboid movement is not clearly understood.

?   Changes in the arrangement of which cytoplasmic structure(s) are most likely to cause the changes in viscosity of the endoplasm associated with amoeboid movement?

Amoebae feed by phagocytosis. Pseudopodia form around a food particle, encasing it in a food cup lined by the amoeba's cell membrane. When the food particle is completely enveloped, the food cup membrane is separated from the external membrane and forms a food vacuole in the endoplasm. Lysosomes, containing digestive enzymes, fuse with the food vacuole and the food particle is slowly digested.

Some species of protistan groups other than Rhizopoda also use amoeboid movement and feed by phagocytosis. Furthermore, certain types of white blood cells in vertebrate organisms engulf bacteria and viruses by amoeboid phagocytosis.

❑   Observe fixed, stained whole mounts of *Amoeba proteus* on a prepared microscope slide to see and identify various organelles.

❑   Observe a live preparation of *A. proteus* using dark field microscopy.

FUNGUS-LIKE PROTISTS

The fungus-like protists resemble fungi in appearance and lifestyle, but the similarities are the result of convergent evolution. Some taxonomists place the four phyla in the Kingdom Fungi, and in fact these phyla are usually included in mycology courses.

Plasmodial Slime Molds, *Physarum polycephalum*

The Plasmodial Slime molds are one of two groups of slime molds presented in Campbell and Reece (2008). Reproductive structures of slime molds, appearing like a crusty dried foam, can often be seen on the bark mulch around campus during and after damp weather.

❑   Obtain a culture plate with *Physarum polycephalum* and observe the specimen with a dissecting microscope.

The vegetative (non-reproductive) stage of *Physarum polycephalum* appears to be a giant, yellow amoeba. Actually, the organism is a **coenocyte** - a body containing many nuclei in a mass of cytoplasm surrounded by a single plasma membrane; the nuclei are not separated into distinct cells. The body of *P. polycephalum* is called a plasmodium which moves by cytoplasmic streaming like that in amoebas.

❑   Place the petri dish containing *P. polycephalum* onto the stage of your compound microscope. Using the 4X objective and maximum light intensity, look for cytoplasmic streaming within the strands of the plasmodium.

With an adequate food supply and favorable environmental conditions, a plasmodium of *P. polycephalum* can grow indefinitely. As it spreads over its substratum, it engulfs small food particles. The substratum in the petri dish is oatmeal agar on which fungi and bacteria grow. Under unfavorable conditions, a plasmodium may transform itself into spore-bearing reproductive structures. The spores formed in the reproductive structures are single cells, each with a cell wall and a nucleus. Spores are expelled from the reproductive structure. If conditions are suitable, each spore may shed its cell wall and become a wall-less, flagellated swarm cell. Two swarm cells can fuse (like two gametes in fertilization). The nucleus of the resulting fusion cell undergoes many mitotic divisions to form a new coenocytic plasmodium.

PROTISTS IN A HAY INFUSION CULTURE AND POND WATER

❑   After you have studied all the organisms mentioned in this chapter, prepare a sample of pond water or hay infusion culture for microscopic study. Each group or students should have at least one sample from each environment.

A hay infusion was prepared by boiling hay in water for a few minutes. This infusion was allowed to stand uncovered for a few days. During that time bacteria in the air fell into it and multiplied in the broth. The infusion was then inoculated with a small amount of soil or pond water, and organisms present in the inoculum multiplied by using the bacteria as a source of food.

The pond water used is a plankton net sample of a small local pond. Plankton are small floating organisms. A fine meshed, cone-shaped net was pulled several meters through the water. Small organisms caught in the net were washed into a small container at the narrow end of the net and removed.

Procedure for preparing hay infusion culture sample

Use coverslip spacers for this preparation (see p. 107). Thoroughly mix the hay infusion culture and place two drops of it on a slide with a methyl cellulose ring. Cover the preparation with a coverslip and observe the organisms in it.

Procedure for preparing the pond water sample

Thoroughly mix the pond water sample and dispense enough of it to just cover the bottom of a <u>clean</u> small Petri dish. Move the mechanical stage of your compound microscope away from the stage opening and place the Petri dish over the stage opening. Observe the sample first with the 4X objective and then with the 10X objective. <u>Do not use the 40X objective</u>. Watch from the side when you change objectives.

❑   Make sketches of at least three different <u>unicellular</u> organisms seen in the hay infusion culture or the pond water, and give the name of the group in which you think each should be placed. Refer to the diagrams, photographs and identification keys available in the lab room to help you identify organisms you have not seen before.

Both the hay infusion culture and the pond water are likely to have many small multicellular algae and animals such as nematodes (roundworms) and rotifers. Rotifers are about the same size as many protists.

You may see several different kinds of green flagellated unicellular organisms alone or in colonies. Most of these organisms are in the clade Chlorophyta (green algae).

CLEAN UP

Lower the microscope stage and remove the specimen(s) from it. Wipe the objective lenses <u>with lens paper</u> to remove water; cover the microscope and return it to the proper spot in the cabinet.

Place used coverslips in the USED COVERSLIPS container by the sinks, and thoroughly wash and rinse the glass slides.

Return the pond water sample to its container and wash and rinse the Petri dish.

REVIEWS AND NEW VIEWS OF PROTISTS STUDIED

Your lab instructor will project photo- and electron micrographs of various protists you have studied.

REFERENCES AND SUGGESTED READINGS

Campbell, NA.; Reece, JB. Biology. 8th ed. San Francisco, CA: Pearson-Benjamin/Cummings; 2008.

Margulis, L.; Schwartz, KV. Five kingdoms. 2nd ed. New York: W. H. Freeman and Co.; 1988.

Wodsedalek, JE.; Lytle, CF. General zoology, laboratory guide. Dubuque, IA: Wm. C. Brown, Publ.; 1963.

Table 6.1. Summary of observed protists.

Protist Genus	Major Clade	Locomotory Structure(s)	Chloroplasts present (?)	Feeding structure(s)	Contractile vacuole present (?)	Other distinguishing feature(s)
Amoeba						
Actinosphaerium						
Paramecium						
Stentor						
Euplotes						
Trichonympha						
Vorticella						
Peridinium						
diatoms						

Protist Genus	Major Clade	Locomotory Structure(s)	Chloroplasts present (?)	Feeding structure(s)	Contractile vacuole present (?)	Other distinguishing feature(s)
Euglena						
Peranema						
Volvox						
Physarum						

CHAPTER 7 - FETAL PIG DISSECTION

LABORATORY SYNOPSIS

This two-session laboratory gives you the opportunity to dissect a pig fetus as an example of mammalian morphology as well as of several accommodations to uterine life. The major role of the cardiovascular system in the integration of the organs is readily apparent by way of dissection.

A secondary emphasis will be placed upon the female reproductive tract. The major components of a female mammalian reproductive system will be seen in the preserved uterus and ovaries of a pregnant sow. Placental and fetal characteristics will first be examined in the intact uterus, and then a single fetal pig in its chorionic vesicle will be removed from the uterus so that you may study the structure of its extra-embryonic membranes and their function in regulating the development of a mammalian embryo.

READING ASSIGNMENTS (these should be done before the lab period begins)

In Campbell and Reece (2008):

Female and Male Reproductive Anatomy	p. 1003-1018

You may want to review the major mammalian systems in Campbell and Reece (2008) as well (skim only):

Digestive	p. 884-893
Circulatory	p. 903-911
Lungs	p. 920-923
Kidneys	p. 962-963
Brain	p. 1070-1084

LEARNING OBJECTIVES

Upon completion of the dissection you should

1. understand the organization of the mammalian heart and its connections with important anterior and posterior structures.

2. understand the importance of the liver as the primary processing center for nutritive materials acquired from the placenta (fetus) or from the gut (adult), and how such items arrive at the liver.

3. know specific circulatory adaptations of mammalian fetuses for life in the uterus, especially the foramen ovale, the <u>ductus venosus</u> (liver shunt), <u>ductus arteriosus</u> (aortic shunt), and the umbilical vessels.

4. be able to identify the major structures of the digestive, urinary, respiratory, and circulatory systems and know their functions.

5. know the function of a placenta and the maternal and embryonic structures of which it is composed.

6. know the parts of the mammalian female and male reproductive system and their functions.

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Revised June 2010
Mark A. Sarvary

FETAL PIG DISSECTION

INTRODUCTION

Everyone ought to have some idea of how they are put together. Morphology can be learned in several ways, through the study of development, or of comparative anatomy for example, but the quick route is via dissection. We cannot provide human beings for you to investigate, but since humans are mammals and most mammals are basically the same, we are letting you loose on the fetal pig. This is a uterine mammal whose embryology is complete, and whose growth before birth is well advanced (gestation length is 17 weeks). We choose the fetal pig for several reasons: it is easy to dissect (fetuses have no fat to speak of), it is economical and easy to come by, and the supply for teaching purposes does not have to be 'engineered' in any way (short of use in teaching, most fetal pigs end up in pet food - at least in the States). In this double-laboratory session, you should get an appreciation of the morphology of the alimentary tract and its associated glands, the respiratory and urogenital systems, and the circulatory system that ties all the systems together. Some careful drawings and notes should stand you in good stead if you eventually go on to medical school and have to suffer through Gross Anatomy.

A note about dissection

Your dissection materials includes razor blades and two pairs of scissors. Cutting is the least of dissection, most of which involves use of a seeker (or probe) and a pair of forceps. *One looks for things rather than excising them.*

As you go through the dissection, thoughts of function should be passing through your mind as you examine the form. Given a blood vessel, for example, the kinds of questions that arise are: "In which direction is the blood flowing? Is the blood in this vessel normally oxygenated? What is the nutrient content of the blood? Is this vessel important for hormonal signaling?" And so on.

The circulatory system has been injected to try and make the arteries red and the veins blue; don't count on complete success!

The externals are pretty unexciting

A surface examination does not teach you much more than you already know. The position of the umbilical cord is obvious. Is your animal a male or a female; how do you know? Does the fetus have mammary papillae (nipples), open external nares (nostrils), and a pinna (external ear)?

The oral cavity and pharynx

Cut back through both sides of the mouth, using a medium scissors and following the angle of the jaw. Along the roof of the mouth, identify the hard palate and soft palate and the opening of the nasal passages in the nasopharynx. Note the glottis, or opening to the larynx, and its protective flap the epiglottis. Probe past the glottis to find the slit-like opening to the esophagus. What is the function of the epiglottis?

Make a transverse cut across the snout about 2 cm back from the tip. What is the function of the extensive folding of the lining of these nasal cavities?

The gut (alimentary tract and associated glands)

Feel for the breastbone (<u>sternum</u>) and use the fine scissors (tips pointing up) and forceps to make a cut between it and a half-inch in front of the umbilical cord (see Figure 7.1). 'Front' is anatomically ambiguous: if you mean the tip of the head the word you need is <u>anterior</u>, but if you mean the belly (the 'front' of a human) use <u>ventral</u>. Anyway, continue the cut <u>posterior</u> (i.e. toward the tail, posterior is the antonym of anterior)*on each side of the cord* to leave a half-inch strip of body wall in the midline. Feel with your finger for the <u>pubic symphysis</u>, a bridge of bone running transversely as part of the pelvis. The cut on each side of the cord should stop just anterior to the symphysis. So far, so good.

Find the most anterior mammary papilla and make a 2-inch lateral cut through the body wall to the left and right of your median incision. Grab the cut body wall with the forceps and lift as you do this (to avoid cutting anything internal to it). Do the same to the body wall just anterior to the pubic symphysis.

Deflect the body wall flaps slightly to see what is internal to the umbilicus itself. You should see a large vein (blue) running from the cord over on the left side and disappearing into the <u>liver</u>. This is the <u>umbilical vein</u> which joins the <u>placenta</u> (removed) to the liver.

? Is the blood in the umbilical vein, in life, oxygenated or deoxygenated? Does it carry little or a lot of excretory waste? Which way does the blood flow? Is this a permanent or only a developmental vein? Does it have valves? Is it nutritious? Is the blood in it like the maternal blood, i.e. containing enucleate RBCs with adult hemoglobin (i.e., hemoglobin A)?

Tie a cotton thread ligature around the vein internal to the umbilicus for marker purposes and then cut the vein between the ligature and the umbilicus. You can now cut the cord transversely *outside* the body to create a fresh cross sectional surface. The large umbilical vein should be visible again, making up a point of a triangle otherwise pointed by two thick-walled blood vessels - the <u>umbilical arteries</u>. Just outside the triangle look for a small and thick cord of tissue; this is the <u>allantoic stalk</u>, a remnant of the embryonic excretory system.
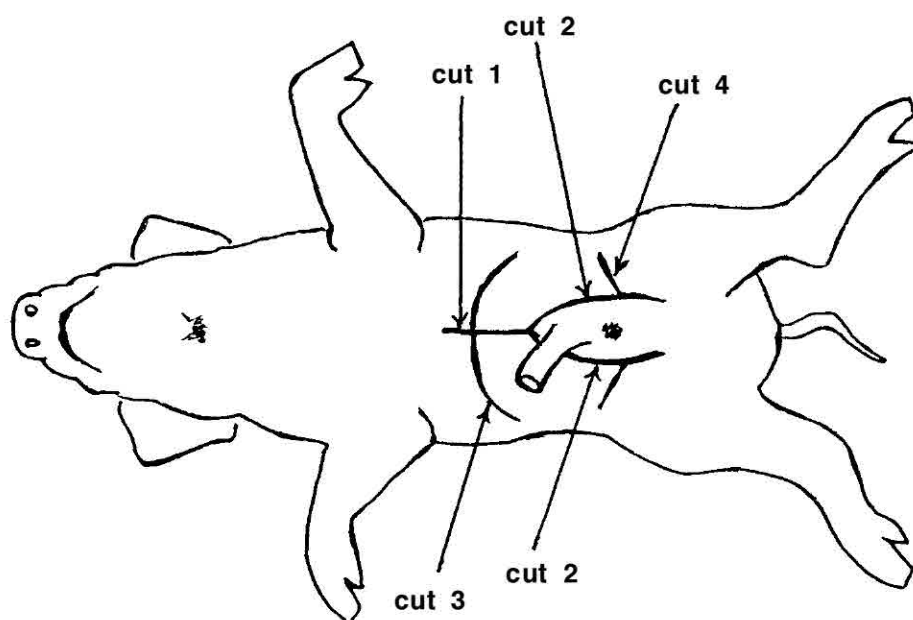
Figure 7.1. Diagram of the location of the initial cuts necessary to expose the viscera.

Now you can deflect the flaps of the body wall and bend the ventral flap of wall posterior to the umbilicus backwards toward the tail (see Figure 7.2). You are looking into a <u>coelom</u> (actually the <u>peritoneal coelom</u> or, more popularly, the <u>abdominal cavity</u>). Note that the coelom is a true *cavity* - in life it is a space. Inside are the organs or <u>viscera</u>, suspended from the back wall of the coelom (the <u>dorsal</u> wall; dorsal is the antonym of ventral) by membranes, or <u>mesenteries</u>.

The anterior wall of this coelom is the <u>diaphragm</u>. Find the <u>liver, stomach, spleen, small intestine (ileum)</u>, <u>large intestine (colon)</u> and the <u>rectum</u>. Use the seeker/probe to lift the right side of the liver anteriorly and find the <u>gall bladder</u> and its <u>cystic duct</u>. The duct joins the gut at the <u>duodenum</u>. The duodenum is U-shaped and its arms encompass the <u>pancreas</u> (which is whitish and lies transversely in the coelom). If you separate the intestines a bit, you will see the <u>mesenteric blood vessels</u> within the mesentery, which otherwise has numerous whitish spots - some of these are little fat islands, but others are <u>lymph nodes</u>. (Figure 7.2 may be helpful. Pages 884-893 in Campbell and Reece (2008) are relevant).
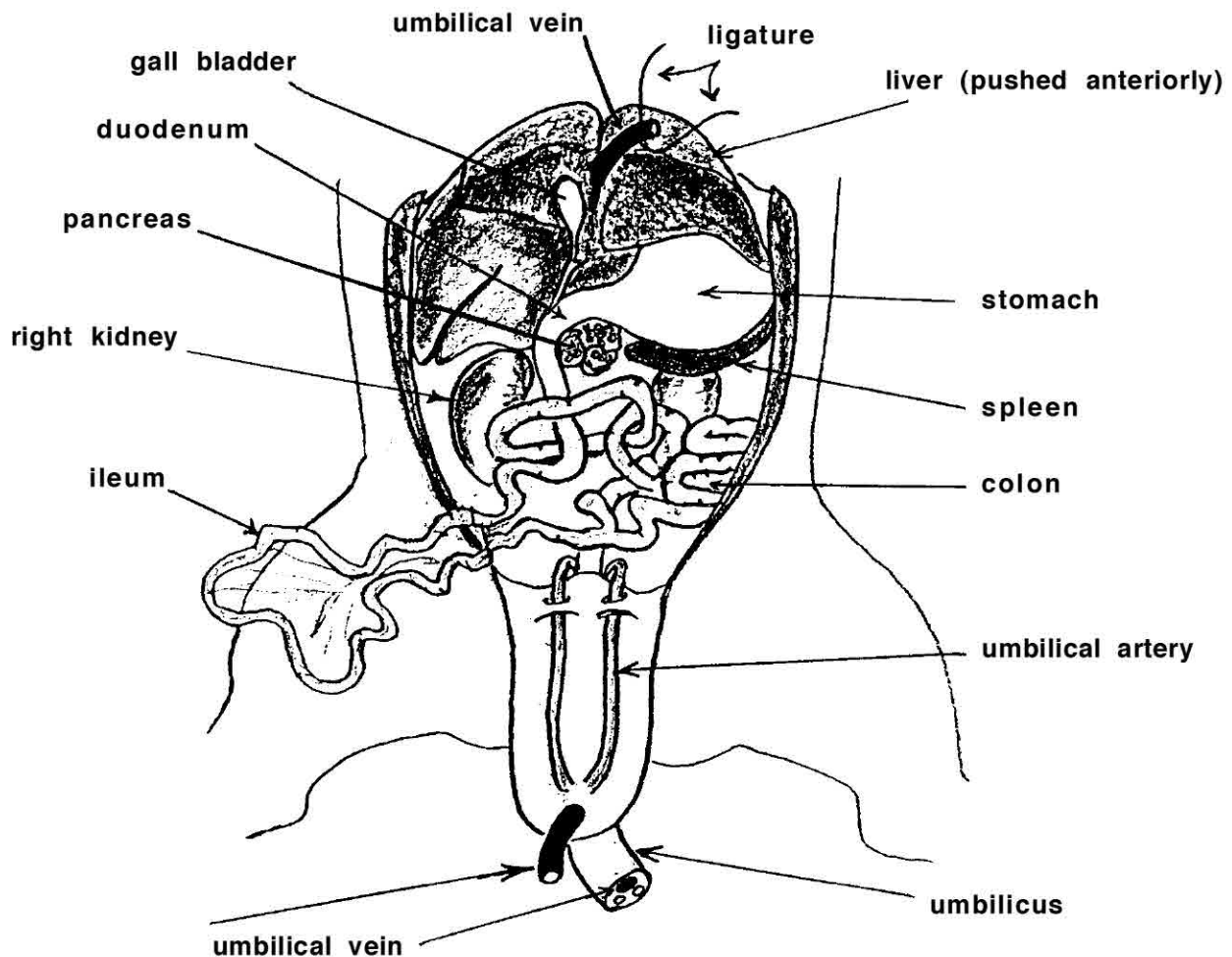
Figure 7.2. Drawing of the principal organs of the peritoneal coelom.

The thorax contains two parts of the coelom

Cut along the midline from the anterior end of your first cut toward the head until you get to the first rib. *Do not cut the first rib.* Spread the thoracic wall to see inside (if there is a lot of resistance, find the diaphragm and make lateral cuts just anterior to it). You should be able to see the heart lying in the pericardial cavity (which is another part of the coelom). If you cut the pericardial wall to the left and right of the heart, you will be able to open the pleural cavities (also part of the coelom). You can now lift the first rib with the forceps, looking to see what you are doing, and cut through the midline symphysis of the first rib. If you do not cut carefully, you will ruin the blood vessels immediately anterior to the heart.

The anterior blood vessels need dissection skill (Figure 7.3)

Remove the pericardium covering the heart itself. Remember that the animal is lying on its back so that left and right are reversed. The heart has two prominent ventricles (see Figure 42.6, p. 903, Campbell and Reece, 2008) and two less prominent atria lying on the anterior side of the ventricles. From the right ventricle, the thick blood vessel running anteriorly is the pulmonary aorta. It curls over a thick blood vessel projecting anteriorly from the left ventricle: this is the systemic aorta.

(The heart is essentially composed of two pumps, one on the left and the other on the right side of the heart. The right atrium and ventricle constitute a pulmonary pump, the atrium collecting blood from the body and the ventricle pumping it to the lungs. The left atrium and ventricle form a systemic pump, the left atrium collecting blood from the lungs and the ventricle propelling it to the body.)

Push the heart to the animal's left to see the venous input to the right atrium. From the head the blood is carried to the atrium in the anterior vena cava. From the trunk the blood is carried to the atrium via the posterior vena cava.

Follow the anterior vena cava as it extends toward the head. Use the probe and cut with care, separating the ventral glandular tissue (which is actually the thymus, a gland of immunological significance) just enough to follow the course of the vein. Several minor veins are received from the neck, but attention should be focused on two principal veins that join together to form the single vena cava. These veins are the jugular veins and they are transporting blood from the brain. At the point where they join, the subclavian veins from the forelimbs also join the anterior vena cava.

If you now push the heart over to the animal's right, you should see the pulmonary veins entering the left atrium. *These blood vessels are rarely colored by the injection medium.*

Find the pulmonary arch again and follow it anteriorly with gentle dissection until it branches into two. The left branch, the left pulmonary artery, is easy to see, especially if you nudge the heart to one side. The right branch ducks posteriorly behind the heart and is lost to view: do not follow it yet.

Now transfer your attention to the systemic aorta. It leaves the heart anteriorly but then swings in a tight arc over to the left and then posteriorly as the dorsal aorta. Look for the short but robust connection between the pulmonary aorta and the systemic aorta known as the ductus arteriosus. This shunt is embryologically important but shuts and degenerates after birth (in human beings, failure to do so is responsible for one class of so-called 'blue babies'). Another embryonic connection is the foramen ovale which connects the right and left atria.

As the systemic aorta is swinging through its arc it gives off two prominent arteries. Find these roots first and only then cut through the anterior vena cava at the level of the roots and deflect the part of the vein running in the neck to see where the arteries are going.

VENOUS SYSTEM

external jugular vein
internal jugular vein
thyroid gland
subclavian vein
anterior vena cava
pleural cavity
right atrium
left lung
posterior vena cava
left ventricle

ARTERIAL SYSTEM

trachea
right carotid artery
right subclavian artery
brachiocephalic artery
anterior vena cava (cut)
posterior vena cava
external jugular vein (cut)
subclavian vein (cut)
left subclavian artery
systemic arch (aorta)

Figure 7.3. The blood system anterior to the heart of the fetal pig. The blood system anterior to the heart of the fetal pig.

The first artery to be given off the systemic arch is the brachiocephalic artery. It runs forward a short distance and then breaks into three arteries. Two of them run anteriorly and side-by-side: these are the carotid arteries. The trachea to the lungs lies between the carotids. The third artery goes off to the right (to the right forelimb in fact); this is the right subclavian artery. (If you did not ruin the jugular veins when deflecting the anterior vena cava, you will find that the carotid arteries run closely attached to the jugulars, and if you look into the joining tissue you should see a white nerve running along with them. This is the vagus nerve, which is actually one of the cranial nerves emanating from the brain.)

The second artery to be given off the systemic arch is the left subclavian artery to the left forelimb. (This arrangement of the arteries in front of the heart is peculiar to mammals. In birds everything is reversed in comparison. And reptiles resemble frogs in having everything symmetrical. The whole matter has embryological and evolutionary significance).

Some blood vessels posterior to the heart supply/drain the gut (Figure 7.4)

With the blood vessels exposed anterior to the heart, it seems logical to delve into the respiratory system. However, finish up the gut anatomy first.

The posterior vena cava (recall!) enters the right atrium after passing through the diaphragm. On the posterior side of the diaphragm it emerges from the liver (difficult to see now; do not break the liver away from the diaphragm in an attempt to see it). Obviously the blood that the vena cava carries must come, in part from the liver, and since the umbilical vein enters the liver most of this liver blood must have come from the placenta. But venous blood from the gut must also get into the vena cava, and it does so via the liver, getting there from the gut by way of the hepatic portal vein - the next vein we have to find.
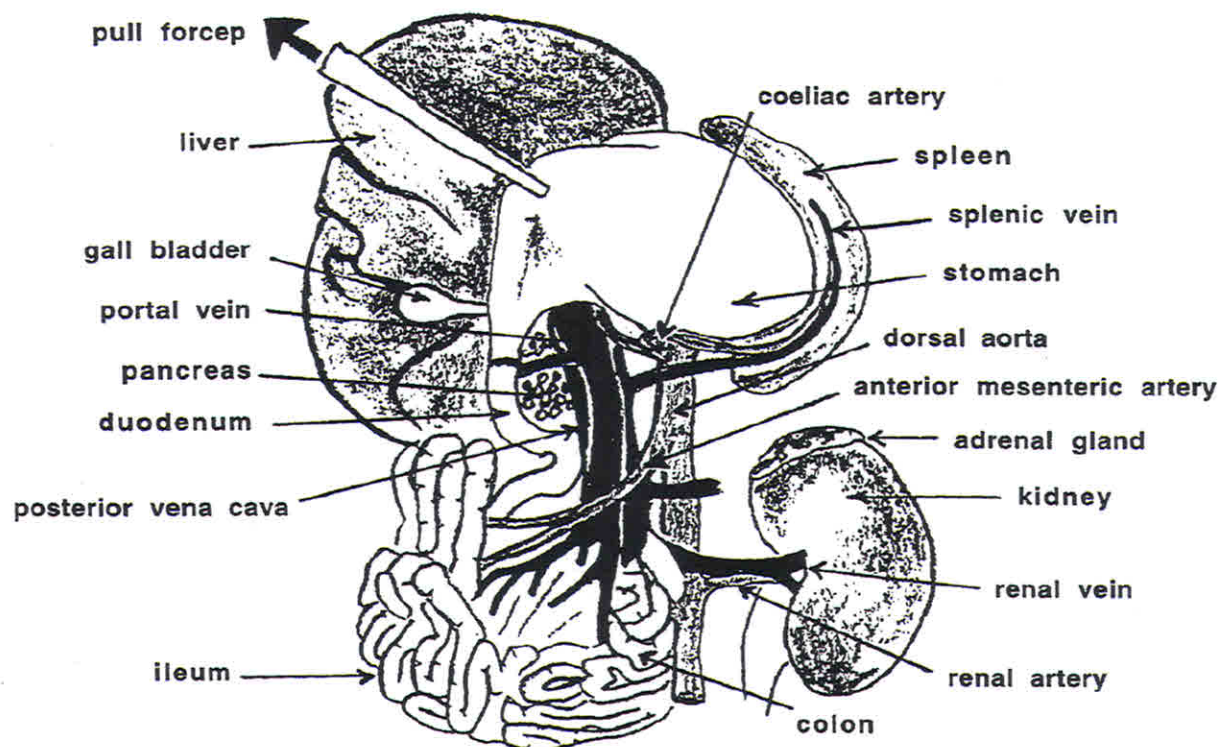
Figure 7.4. The blood supply to the gastrointestinal tract of the fetal pig.

Push the viscera over to the animal's right so that you can cut away the diaphragm on the left side and follow the dorsal aorta as it runs posteriorly. If you are careful enough, you will see the coeliac artery going to the stomach and spleen, and then the anterior mesenteric artery going to the intestines. The mesenteric artery breaks up into many smaller arteries that fan out throughout the gut mesentery. The smaller arteries run side-by-side with small veins that coalesce to form the hepatic portal vein passing from the gut and passing to the liver, entering it at a point fairly close to where the umbilical vein enters it (Figure 7.4).

With forceps and seeker/probe begin to chip away the substance of the liver. Take your time. You want to see what happens after the umbilical and portal veins enter the liver (they join to form a wide channel - the ductus venosus) and how their blood gets into the vena cava (the ductus venosus joins the vena cava). (After birth, the umbilical vein degenerates to a solid cord of tissue, as does also the ductus venosus. All portal blood is subsequently passed through liver capillaries before entering small liver veins that join the persistent vena cava. The alimentary importance of this situation should not be overlooked.)

Preparing to close up for the day: excision of the gut

You can now cut through the rectum and, turning the viscera to the side, free the intestines by cutting through the dorsal mesenteries. Take care not to damage the kidneys, or the dorsal aorta, or the posterior part of the posterior vena cava in doing so. Cut through the esophagus where it enters the stomach and begin to remove the stomach and spleen by cutting their common mesentery, but be careful to leave intact the posterior vena cava and the dorsal aorta. The whole gut and associated glands should eventually come free. *It goes into the specially marked container for subsequent disposal.*

❑   Clean Up:

1.   Cover your fetal pig for the night.

2.   Wash your instruments with warm soapy water, rinse them with tap water and dry them thoroughly. Pay special attention to joint of the scissors.

3.   Wash your hands thoroughly with plenty of soap and water.

****   ****   ****   ****

The return to the thorax

Take the scissors and, leaving a short identifying root in each case, cut the brachiocephalic artery and the left subclavian artery. Cut the pulmonary arteries just anterior to their origin from the pulmonary arch, and then turn the heart to the left to sever the posterior vena cava. There are still a pair of short veins entering the left atrium; these pulmonary veins should also be cut. The heart should now be ready to lift out of the thorax.

Look at its dorsal side critically, like a heart surgeon (Figure 7.5). The major blood vessel origins should still be apparent.

Slit open the right atrium and note that there is a vertical banana-shaped opening from the right atrium into the left atrium (Figure 7.5). This is the foramen ovale (oval window') by which blood is shunted directly from the right to the left side of the heart without being pumped to the lungs (which are unexpanded and are resistant to the passage of blood): the foramen ovale shuts with the first breath of the new-born, and if it does not, the defect is the cause of another class of 'blue baby' in human beings.

If you now slice the heart horizontally, you may be fortunate to see the atrioventricular valves (tricuspid and bicuspid) and the aorticopulmonary septum or wall separating the pulmonary and systemic trunks. If you do not succeed, put it down to ham-fistedness and see if a neighbor has had better luck.

left subclavian artery — brachiocephalic artery

systemic arch

pulmonary arch — anterior vena cava

dorsal aorta (root)

Pulmonary veins — right atrium

left atrium — posterior vena cava

A. DORSAL ASPECT

left ventricle

left atrium — cut edge

right ventricle

systemic arch — foramen ovale

brachiocephalic artery — cut edge

left subclavian artery

pulmonary vein — posterior vena cava

anterior vena cava

B. RIGHT LATERAL ASPECT

Figure 7.5. (A.) The dorsal side of the heart. (B.) The view from the right lateral side of the heart.

Removal of the heart reveals the lungs

Returning to the corpse, the trachea can now be seen separating into the bronchi, which terminate in the lungs. Note that the lungs are unexpanded. The trachea are supported by prominent and transverse rings of cartilage. Check the number of lung lobes to left and right, equal or unequal?

The 'uro' of the urogenital system refers to kidneys

Now that the gut has been removed, the left and right kidneys are easy to see. Note that the dorsal aorta supplies them with a pair of renal arteries, while the posterior vena cava drains them by a pair of renal veins. Note the adrenal gland on the anterior apex of each kidney (Figure 44.14, p. 963, of Campbell and Reece, 2008, is relevant).

From the midline side of each kidney there extends posteriorly a ureter. This is the excretory (urinary) duct of the kidney.

To see more you will have to deflect the flap with the umbilicus posteriorly, and when you do this you should see those umbilical arteries previously seen in the umbilical cord section arising from the dorsal aorta. The ureters run parallel to the arteries and then apparently get lost in the mass of tissue at the base of the flap. This tissue contains the bladder, which is otherwise connected to the cord of the allantoic stalk that was also seen in the umbilical cord.

To go on from here demands different techniques according to whether your fetal pig is a male or a female. If you are still not sure about the sex, look for a dark pit just posterior to the umbilicus. If it is there, a penis is hidden inside and you have a male. And if it is not there, presumably your fetal pig is a female.

The female genital system is somewhat easier to dissect than the male (Figure 7.6)

Spread the hind legs as best you can and insert a blunt probe into the urogenital orifice, passing it forwards until resistance to further passage is felt. Now take your coarse scissors and cut through the pubic symphysis about a 1/4 inch on the left and right sides. Carefully remove the center section.

Posterior to the kidneys lie the ovaries , each of which is attached to the dorsal side of the peritoneal cavity by a mesentery. Along the edge of each ovary look for the Fallopian tube (oviduct). The opening of the Fallopian tube is located on the upper anterior edge of the ovary (incidentally, the opening is the only opening from the exterior into a coelomic space). Posteriorly, each oviduct widens to a coiled uterine horn and then, more posteriorly, the two horns fuse as the uterus. This then narrows down as the vagina which is confluent with the urogenital sinus (also variously known as the vestibule or vaginourethral passage).

?   Is this uterine set-up any different from the human being (refer to Figure 46.10, p. 1004 in Campbell and Reece, 2008)? At which points in the female reproductive anatomy of the human female is contraception possible? What is the difference between contraception and sterilization? What is the least invasive method of physical sterilization?

Urethral exposure of the male requires patience (Figure 7.7)

The principal objective is to keep the urinary duct from the bladder to the exterior, the urethra intact. So probe with a finger for the position of the pubic symphysis and to locate the scrotal sacs. Now cut through the soft tissue over the pubic symphysis, but do not continue forward. Instead, locate the penis again in the median strip of abdominal wall, free it from surrounding tissue (try and keep the bladder intact) and then push it to one side.

Cut through the pubic symphysis about 1/4 in. to the left of the midline, using your coarse scissors. Now, keeping an eye on the positions of the penis, scrotal sacs and where the urethra is lying in the midline, lift the cut edge of the pubic symphysis and chip away the soft bone toward the right side, so that the urethra can be exposed along it entire length.

Note that the urethra runs pretty parallel to the rectum (the thin tissue between them is a remnant of the embryological process in which each of them was originally derived from a single chamber, the cloaca, which is the common exit for both urogenital and alimentary waste products in all vertebrates inferior to the mammals).

Look to where the genital ducts, the vasa deferentia, are curling dorsally over the ureter of their side in the abdominal cavity and entering the base of the urethra. At this entry point lies a pair of small glandlike receptacles: these are the seminal vesicles. Between them is sometimes noted a very small, beadlike prostate gland (but this gland is really only large and prominent in aging mammals). A pair of prominent glands however, are located further along the urethra, these are Cowper's glands. (The secretions of the vesicles, prostate gland and Cowper's glands collectively form the seminal fluid.) (Figure 46.11, p. 1005 in Campbell and Reece, 2008, shows these structures in humans.)

Posteriorly, the urethra bends ventrally and anteriorly as the penile urethra within the penis. Don't confuse Cowper's glands with the cavernosa muscle of the penis itself. A cross-section cut along the penis will reveal the presence within it of the urethra, as well as a pair of corpora cavernosa (spongy tissue involved in the erection process).

? In the human male, what is the essential difference between sterilization by castration and vasectomy? What effect does each method have on a) ability to perform the sexual act, b) ability to produce a secretion, c) orgasmic muscle contraction? Is sterilization by either method immediate?
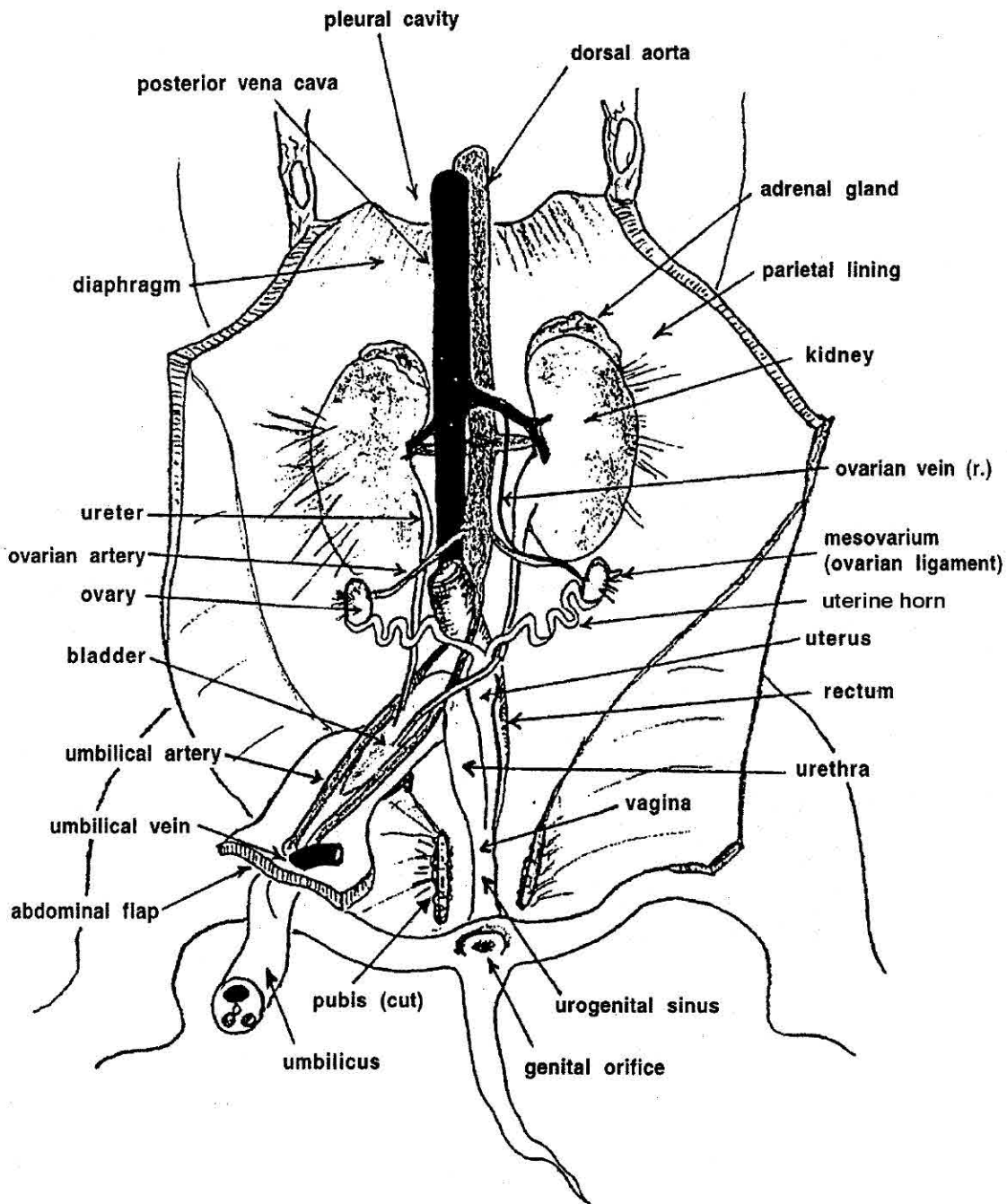
Figure 7.6. Urogenital system of the female fetal pig.
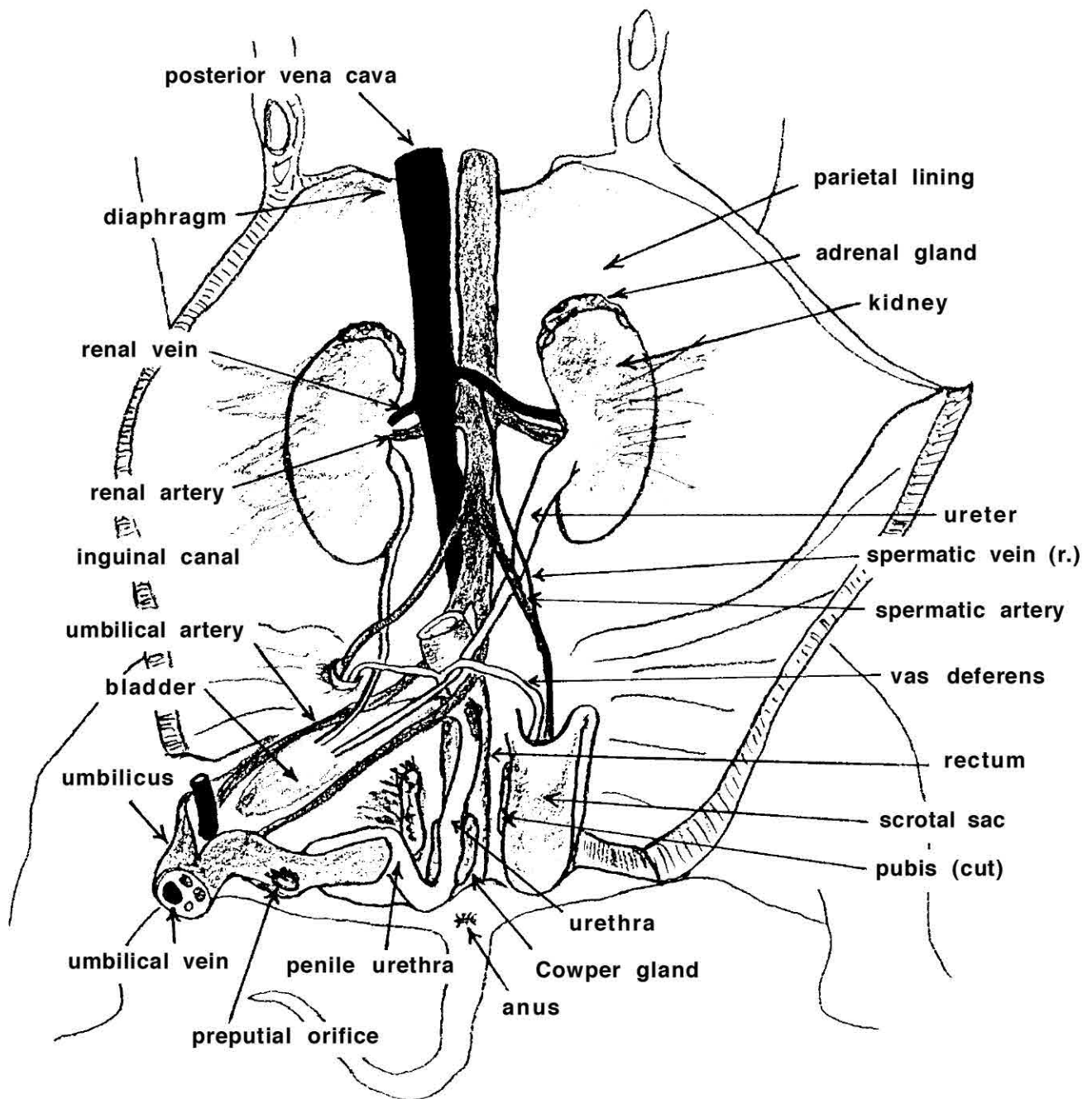
Figure 7.7. Urogenital system of the male fetal pig.

If time permits . . .

The following are purely optional exercises, but with a dissected fetal pig in front of you if you have any extra time and interest you might wish to take a look at one or more of the following. (Remember, none of what follows is required for this course.)

The Brain

If you are EXTREMELY careful you might be able to cut away the thin cranial bones and expose the brain of the pig. The brain tissue is very soft and easily damaged. The trick here is to make very short cuts of the bone with the scissors as you go and so avoid cutting into the underlying brain with the scissor tips. By cutting these bones into plates they can then be peeled gently off with tweezers. Notice that the brain is covered by a membrane and is in its own coelomic cavity. The fluid in the spine is continuous with the fluid in this brain cavity.

? Compare the tissue strength of the brain to that of the liver. Which is tougher? The head bones may be thought to be an exoskeleton for the brain. What effect does this have on the toughness of the organ these bones surround?

See if you can find the **cerebrum**, a part of the forebrain, and the **cerebellum**, a part of the hindbrain. A careful (and lucky!) medial longitudinal cut down through the brain might let you see the interior midbrain as shown in Figure 49.14 on p. 1074 of Campbell and Reece (2008). You can try to see where the **spinal cord** meets the expanded pons of the midbrain. Anterior to them you might look for the **thalamus** and hypothalamus of the forebrain.

Peripheral nerves

The brain and spinal cord make up the central nervous system. The **peripheral nerves** makes up the rest. A good place to look for peripheral nerves is along the spinal column. To do this you will have to remove the ventral viscera from the abdominal and thoracic cavities. When the spine is exposed look to the right and left of it to find white-colored cords extending out of it and running parallel to it along its lengths. These are peripheral nerves. Some may group together into ganglia which are part of the **sympathetic nervous system**. (Figures 48.14 and 48.15, on p. 1057 of Campbell and Reece 2008, may give a good perspective here.) Many nerves will extend from the spinal column to extend into the forelimbs and into the hindlimbs. See if you can find some of them.

Lungs

Excise one lobe of a lung from your pig. Since it is a fetal pig its lungs will not be fully developed. However, use a sharp razor blade to make a clean section through the excised lung lobe. Examination of this lobe under a dissection microscope may reveal **bronchioles** and **alveoli** (see Figure 42.24, p. 919, of Campbell and Reece 2008).

Kidneys

Excise one of the kidneys of the fetal pig and through it make a medial longitudinal section (as shown in Figure 44.14, p. 962 of Campbell and Reece 2008). You may see the large **renal pelvis**, from which the **ureter** extends and into which the **collecting ducts** of the **nephrons** delivers urine. Examine your section with a dissection microscope and see if you can find the capillary beds in the **renal cortex** and the extensions of the Loops of Henley into the **renal medulla**.

Vertebrate eye

The eye is surrounded by much connective tissue, by muscles, and by the eyelid. If you carefully cut these away using your fine scissors you may be able to remove the eye of the fetal pig. Then you should be able to find the large **optic nerve** entering at the posterior (see Figure 49.10, p. 1071 of Campbell and Reece 2008). If you section the eye, by cutting into the vitreous humor with the point of the fine scissors and cutting all the way around the eye, then you should be able to see the internal **retina** which contains the light sensing cells. Peel back the retina to expose the underlying bed of capillaries in the **choroid**. See if you can find the **lens** of the eye and the ligaments that attach it to the surrounding **ciliary body**.

REFERENCES

Campbell, NA.; Reece, JB. Biology. 8th ed. San Francisco, CA: Pearson-Benjamin/Cummings; 2008.

Evans, WL.; Addison, EL. The fetal pig: a photographic study. New York: Rhinehard Press; 1958.

Fetal Pig Dissection Worksheet

Name: _____   Section: _____

1. During the process of birth the foramen ovale closes.

    a) Describe how this alters the flow of blood.

    b) Would it be a beneficial or harmful if the foramen ovale did not close? Explain your reasoning.

2. The circulatory system of the fetal pig has several adaptations for fetal life. Aside from the foramen ovale described above, name and describe two other adaptations.

3. How can you distinguish a male fetal pig from a female? Describe all differences (both external and internal).

CHAPTER 8 - ANGIOSPERM STRUCTURE AND FUNCTION

LABORATORY SYNOPSIS

During this laboratory you will study the morphology of angiosperms using live materials, hand-cut thin sections, and commercially prepared thin sections, and relate the structural organization of plants to the processes of photosynthesis, gas exchange, nutrient procurement, and transport of water and nutrients. You will learn techniques for studying water transport in plant shoots and cutting and staining thin sections of plant material for microscopic study. Projected transparencies of photo- and electron micrographs will supplement your study of plant morphology.

LABORATORY OBJECTIVES

Conceptual Objectives

1. Know the vegetative (nonreproductive) organs of a vascular plant and their arrangement in the organism.

2. Know the three basic tissue systems and their general arrangement in the organs of an herbaceous angiosperm, and be able to identify these tissue systems in stained thin sections of the organs.

3. Know the functions of the following structures, tissues and regions in an angiosperm and be able to identify them in live organs and/or stained thin sections of the organs.

epidermis	pericycle	sclerenchyma fibers
root hair	stele	mesophyll
cortex	xylem	vein
endodermis	vessel	bundle sheath
plasmodesmata	phloem	stoma
vascular cylinder	vascular bundle	guard cell
Casparian strip	vascular cambium	subsidiary cell

4. Know the origin of a branch root.

5. Understand the concepts of apoplast and symplast and know the components of each of these systems.

6. Understand how xylem vessels are "loaded" with mineral ions and how root pressure is produced.

7. Understand the transpiration–cohesion–tension mechanism for the movement of water in a vascular plant, and know the main pathway of water movement through a plant by this mechanism. (This mechanism is covered on pages 764-779 in Campbell and Reece 2008.)

8. Be able to explain under what conditions a plant would use the root pressure mechanism, compared to the conditions under which the transpiration-cohesion-tension mechanism would be used. Be able to describe how the same plant could use both of these mechanisms but would have to use them at different times.

Procedural Objectives

1. Know how to make hand-cut thin sections of plant material.

2. Know how to determine stomatal density in an epidermis.

READING ASSIGNMENT (should be done <u>before</u> lab period)

In Campbell and Reece, *Biology*, 8th ed. (2008):

      Chapter 35:   Beginning of chapter to cork cambium& ..   p. 738-754

      Chapter 36:   Beginning of chapter to   p. 764-779
                       Differences in water potential& &

      In *Investigative biology*:
                      This chapter of the lab manual

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

1.   What are the components of a plant's shoot system?

2.   All kinds of plant cells have cell walls called "primary walls." Some kinds of plant cells also have "secondary walls." In a cell with a secondary wall, is the secondary wall inside or outside the primary wall?

3.   What are the three tissue systems of an angiosperm? What are the three organs found in vascular plants?

4.   What is a tissue (in any kind of organism)?

5.   Angiosperms have supportive tissues composed of specialized cells. They also have a "hydraulic" support system. What are the constituents of the hydraulic support system and how does the system provide support?

6.   a.   What are the components of the "apoplast" of a vascular plant?
           b.   What are the components of the "symplast" of a vascular plant?

7.   a.   What is a differential stain?
           b.   What is a potential advantage of a differential stain over a non-differential stain?

8.   To generate root pressure, plants typically actively alter what component of the water potential in their xylem?

                                                    Paul R. Ecklund

                                                    Revised June 2010
                                                     Mark A. Sarvary
                                                      Scott T. Meissner

INTRODUCTION

In this laboratory we shall address the relationship between structure and function in vascular plants with respect to the processes of nutrient procurement, water and nutrient transport, gas exchange and photosynthesis. Much of the background information for this laboratory is in Campbell and Reece (2008). See the reading assignment for this laboratory. The following introductory material summarizes much of the information in Campbell and Reece and supplements it.

Vascular plants, being mainly photosynthetic and terrestrial organisms, are the results of an evolutionary compromise to a dilemma. The overall process of photosynthesis involves the absorption of a gas, carbon dioxide, by plant cells and the subsequent synthesis of organic molecules using carbon dioxide, water, and energy in the form of light. The ideal photosynthetic structure would seem to be one with a large surface area to volume ratio to provide the maximum surface area for the absorption of light and carbon dioxide. But, before the molecules of an atmospheric gas can diffuse through a cell membrane, they must be dissolved in water. This physical requirement dictates that the gas-absorbing surface be kept moist. Since the water potential of the atmosphere is usually lower than that of plant cell walls, water readily moves from the cells into the atmosphere. Consequently, the "ideal" structure for photosynthesis also would provide the maximum surface for the evaporation of water from the plant tissues, a process called **transpiration**. Metabolically active plant tissues must maintain a relatively high water content to remain functional. The photosynthetic structures of terrestrial vascular plants possess an outer, waxy surface layer, the **cuticle**, which inhibits the passage of molecules into and out of these structures. Consequently, the cuticle prevents considerable water loss from these plant surfaces. Gases, such as carbon dioxide and water vapor, pass through cuticle-covered surfaces by means of stomata, special pores whose sizes are regulated by the cells forming them. Whenever a plant takes in carbon dioxide, it loses water through the common passageway. Thus, photosynthesis and transpiration are two interdependent processes of terrestrial plants. The evolution of vascular plants has resulted not only in the development of a vascular system to supply the aerial, photosynthetic parts of the plant with water and nutrients, but also in the development of various morphological and physiological adaptations that enable the plant to photosynthesize rather efficiently and yet conserve water. Transpiration might appear to be a necessary disadvantage that a plant must undergo to obtain carbon dioxide; nevertheless, the vascular plant uses transpiration to its advantage in moving water and nutrients from its roots to its photosynthetic regions with no expenditure of metabolic energy.

The typical vascular plant is composed of two integrated systems–a root system and a shoot system. The main functions of the root system are absorption of water and mineral nutrients and anchorage of the plant to its substratum. The root system is extensively branched to provide a large absorptive surface area. The shoot system consists of the stem, leaves and lateral buds which may form branches. The shoot system also may produce reproductive structures such as cones or flowers. The primary function of the leaves is photosynthesis; however, in most species the younger part of the stem also accomplishes this process. The stem, which may or may not have extensive branching, spatially arranges the leaves for optimum exposure to light, and provides a connection between the photosynthesizing and transpiring leaves and the water- and nutrient-absorbing roots, which require the products of photosynthesis for their growth and maintenance. The connection is accomplished by a continuous system of conduits, the vascular system, from the roots to the leaves.

Conduits of the **xylem** carry water and dissolved substances from the roots to the leaves. The organic products of photosynthesis are transported in the conduits of the **phloem** from the leaves to regions of growth and storage throughout the plant. Although movement of substances in the xylem is mainly "upward" (from roots to leaves), substances are transported both "up" and "down" the plant through the phloem conduits. For example, the growing shoot tip receives its necessary nutrients (both organic and inorganic) via the phloem from the subtending leaves.

Water is most essential for living organisms because it is the medium in which the numerous metabolic processes occur and in which gases move into and out of cells, but water also provides support for the terrestrial plant. The importance of fully turgid cells to the support of the terrestrial plant can readily be seen by the appearance of a wilted leaf or stem. In addition to the hydraulic support system, however, vascular plants have tissues composed of mechanical or supportive cells with rather thick cell walls. The presence of these mechanical cells is an adaptation for living in a terrestrial environment where the buoyant effect of water is absent.

The three main organs of a plant–leaves, stems and roots–are composed of several tissues. A **tissue** is "an integrated group of cells with a common structure and function" (Campbell and Reece 2008). Vascular plants are generally considered to be composed of three basic tissue systems. Each of the tissue systems includes more than one cell type. The **dermal tissue system** provides the outer protective covering of the plant. The **vascular tissue system** consists of the conducting cells of the xylem and phloem and a few other kinds of cells. The **ground tissue system** includes all tissues other than surface and vascular tissues.

Plants also have tissues called **meristems** which are restricted to the regions of growth. The function of meristematic cells is the production of new cells. All the cells of a vascular plant are derived from meristematic cells. We shall address the locations of meristems and their functions in plant growth in a laboratory in Bio. 108 on ANGIOSPERM GROWTH AND DEVELOPMENT. Nevertheless, you will observe meristems in this study.

The term **parenchyma** applies to a cell type and the tissue composed of that cell type. Parenchyma cells are the most abundant cells in herbaceous plants (plants which do not produce persistent, woody stems). Mature parenchyma cells have only thin primary cell walls and are relatively unspecialized, thus they retain the ability to divide and produce new cells. Parenchyma cells perform several functions including the storage of food reserves and photosynthesis.

Three tissue types contribute to the support of stems and leaves of angiosperms. Strands of bundled **collenchyma** cells help support young, growing parts of a plant. Mature collenchyma cells are similar to parenchyma cells by having living protoplasts and only primary cell walls, but the cell walls are much thicker. Collenchyma cells can elongate with the enlargement of the organ they support. **Fibers**, which are long, slender tapered cells with heavy secondary cell walls, compose a tissue called **sclerenchyma**. Fibers are generally in bundles running lengthwise in the stem or leaf. Usually mature fibers do not have living protoplasts. Tracheids and vessel elements, which compose the water-conducting tubes of the **xylem**, also have heavy secondary walls. Bundles of xylem tubes and/or fibers act as supporting "rods" in stems and leaves.

The preceding discussion indicates that a plant may be regarded as a multicellular organism in which the cells are organized into tissues, tissue systems and organs. Another way of viewing the organization of a plant is to regard it as two systems–one "living" and one "non-living." The living system is called the **symplast**, which consists of all the cell protoplasts interconnected by plasmodesmata. The non-living system, called the **apoplast**, is composed of the walls of all the cells, spaces between cells and cavities within the walls of certain cell types. Mature fibers and conducting cells of the xylem lack protoplasts; the cavities inside the heavy walls of those cells are included in the apoplast. The concepts of symplast and apoplast are important in understanding the currently accepted hypothesis explaining the upward movement of water and ions in a vascular plant. The concept map on the next page (Figure 8.1) summarizes the two ways of regarding plant structure.

In this laboratory you will study the cellular and tissue composition and organization of herbaceous angiosperms and relate that structural organization to various processes performed by the plants. You will hand cut thin sections of roots and stems and stain the walls of their cells with a differential stain, toluidine blue. Toluidine blue is called a differential stain because its color depends on the composition of the material to which it binds. Thick cell walls (those with secondary walls) contain a large amount of **lignin**, a

substance which is not abundant in thin primary cell walls. When toluidine blue is bound to heavily lignified cell wall material, it is blue green, but it is red to purple when it attaches to cell walls with little or no lignin. Not all histological stains have this property of color variation depending on the substance to which they attach.

Selected species of angiosperms (flowering plants) will be used to represent vascular plants; however, one should bear in mind that angiosperms are the most recently evolved vascular plants and, therefore, possess certain traits which are not typical of all vascular plants. Angiosperms are divided into two major groups, monocots and dicots, based on the number of embryonic leaves, or cotyledons, possessed by the plant embryos. A monocot embryo has one cotyledon; a dicot embryo has two embryonic leaves. Because of the time constraint, you will study mainly herbaceous dicots.
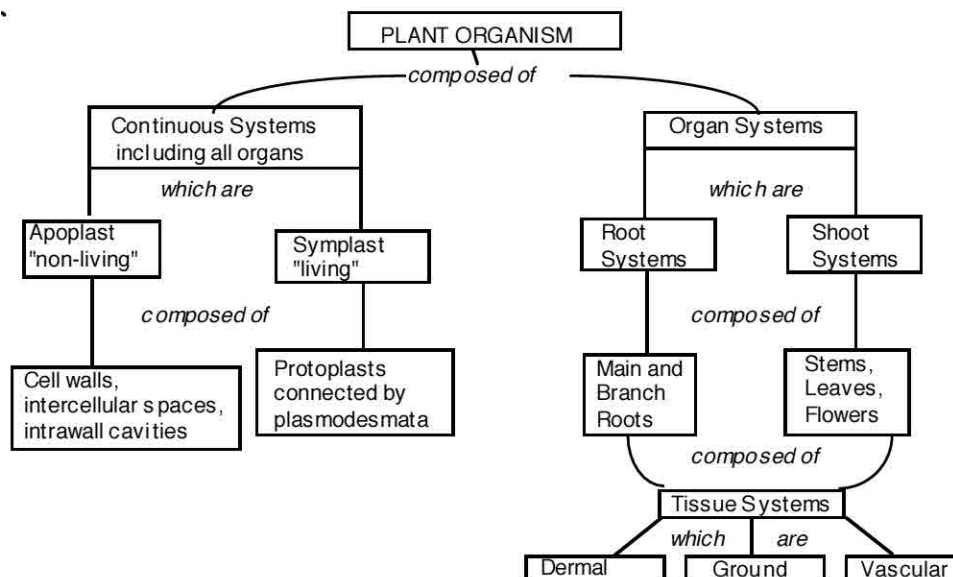
Figure 8.1. Concept map showing two ways of interpreting the structural organization of vascular plants.

ANGIOSPERM MORPHOLOGY

❑   Work individually on these exercises. Be sure you observe all the specimens mentioned in the lab text.

❑   Obtain and prepare the following items.

1 compound microscope

1 dissecting microscope

1 large petri dish bottom or lid

2 flat toothpicks

1 clean microscope slide

1 sharp, single-edge razor blade (if the blade is new, wipe it with a dry paper

towel or tissue to remove grease)

1 small cutting board

1 small petri dish with 50% ethanol (3-4 mm deep)

1 small petri dish with distilled water (3-4 mm deep)

Mark the water dish to distinguish it from the ethanol dish.

toluidine blue solution

1 beaker containing a small volume of water   1 of each for two persons to share

1 plastic bulb dropper

NOTE: TOLUIDINE BLUE STAINS CLOTHING, HUMAN SKIN, BENCH TOPS, ETC.; KEEP ITS CONTAINER ON A PAPER TOWEL OUT OF YOUR WORK AREA UNTIL YOU NEED IT.

External Morphology of the Root System

❏   Carefully remove a pea seedling from the germination box, place it in the petri dish bottom/lid and observe its gross morphology.

The seedling consists of a developing shoot and root system which are using nutrients originally stored in the seed for growth. Pea is a "dicot;" its two fleshy cotyledons are attached to the region of transition from root to stem

❏   Place the dish with the seedling on the black stage plate of a dissecting microscope and observe the root system.
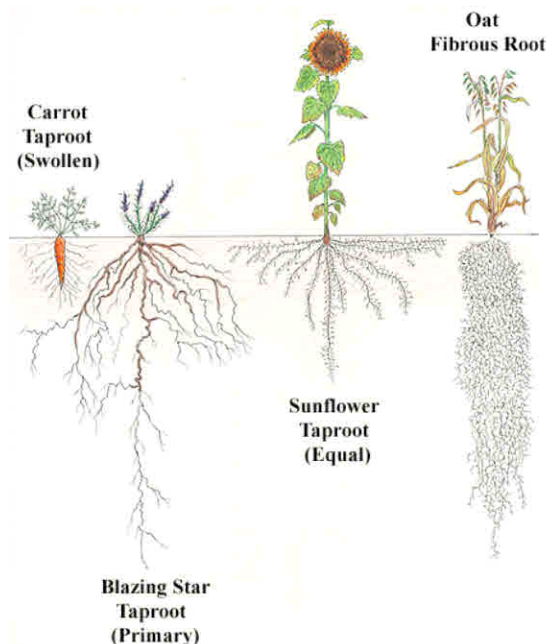
The root system consists of a main root with several lateral, or branch, roots. Each branch root is similar in structure to the youngest part of the main root. The tip of each root is a conical mass of cells, the root cap, which protects the apical meristem from abrasion as it pushes through the soil. The apical meristem is a region of dividing cells which produce new cells for root growth. The first two or three millimeters from the root tip is a region of cell division. Behind this region the cells elongate and then become specialize into the mature tissues of the root. The presence of **root hairs** indicates the region of maturation. A root hair is an outgrowth of the cell wall and membrane of an epidermal cell. Collectively, the root hairs greatly increase the absorptive surface area of the epidermis.

Figure 8.2. Comparative root systems

❏   Observe the root caps and root hairs. When roots are growing in soil the root caps secrete a slimy substance. Can you see a slimy material on any of the root tips?

?   What is a likely function of the slimy secretion of root caps?

❏   Observe a branch root. Note that its epidermis is not continuous with that of the main root; the epidermis of the main root is broken around the emerging branch root.

❑ Look for small bumps on the surface of the main root.

Each bump is a region in which a branch root is developing. Branch roots originate inside the root and grow outward.

Preparation of Root and Stem Cross-sections for Microscopic Study

For efficiency you will cut and stain sections of a root and a stem at one time, even though you will study roots and stems at different times during the laboratory period.

❑ Use the pea seedling from the preceding study and obtain a piece of *Impatiens* sp. stem.

Preparation of Stem Sections

Place the stem piece on the cutting board and make a cross (perpendicular to the long axis) cut through the stem piece a few millimeters from each end; discard the short pieces cut off.

Cut 4 or 5 cross sections (as thin as you can make them) of the stem using the procedures described below.

Preparation of Root Sections

Place the pea seedling on a cutting board. With a razor blade make a cross (perpendicular to the long axis) cut through the main root a short distance from the tip where root hairs are beginning to form and discard the root part with the root tip. Make another cross cut through the main root about 1 cm (1/4 - 1/2 in) from the "seed" and discard the root portion attached to the "seed" and shoot. Cut off branch roots to leave only 2-3 mm projecting from the main root. Cut at least 4 very thin cross sections from each end of the remaining root part using the procedures described below. Include in these sections one or two through regions with branch roots. Attempt to make one of your cuts through the middle of the branch root. Attempt to make another of your cuts through a bump where a branch root is developing.

This procedure gives you root sections from two different regions of development in the root: an earlier stage of development near the root tip and a later stage near the shoot.

Procedure for Making and Staining Hand-cut Sections

1. Using a sharp razor blade, cut cross sections as thin as you can by using a long, slicing motion in one direction (not back and forth) toward yourself. Do not discard partial sections; they may be better than whole ones for viewing.

2. Immediately put each section in the 50% ethanol, and leave it there for at least five minutes. This is a "fixing" solution which kills and fixes the cytoplasm of each cell, but it also has a lower surface tension than water has and reduces the number of air bubbles in each section.

3. Using a flat toothpick, transfer the sections to a slide and put a small drop of toluidine blue solution on each section.

4. Allow the sections to stain for about one to two minutes (not longer). Then transfer them to distilled water.

Anatomy of the Root

❑ Leave the stem sections in distilled water. Put the thin root sections on a clean microscope slide and cover each section with a drop of water.

❑ Observe the sections with a dissecting microscope using a white stage plate; use the highest magnification and highest light intensity possible.

❑ Find sections which have distinct purple (pink) and blue green regions.

Recall from the introduction that toluidine blue is blue green when it is bound to the abundant lignin in heavy secondary cell walls, but is red/purple if little or no lignin is present.

? Portions of which plant system(s) are stained with toluidine blue: apoplast, symplast or both?

? How are the cells with lignified (blue green) cell walls distributed in the root?

? What are the functions of plants cells that produce thick cell walls with much lignin?

❑ Select your best section from each end of the root. Sections from the younger end should have smaller diameters and root hairs on their surface. Place these two sections side-by-side on the slide, and compare them to the one in the projected photomicrograph.

The cross section used for the photomicrograph was from a root of buttercup (*Ranunculus* sp.), and was 5 to 10 μm thick. Your root sections are likely to be 500 to 1000 μm thick. Buttercup, like pea, is a dicot angiosperm. The buttercup root section was stained with four different stains which are relatively specific for certain cellular constituents. See Table 8.1.

Table 8.1. Four histological stains, their colors and cellular components stained by each.

STAIN	COLOR	CELLULAR COMPONENT(S) STAINED
Fast green FCF	Green	Cellulose* in cell walls
		Cytoplasm
Safranin-O	Red	Lignin* in cell walls
		Suberin** in cell walls
		Nuclear material (chromatin, chromosomes and RNA)
Crystal violet	Violet	Starch in amyloplasts
		Nuclear material
Orange G	Yellow to Orange	Cytoplasm
		Its main function is to differentiate the staining intensities between safranin-O and crystal violet in substances stained by both dyes.

* The walls of all plant cells contain cellulose. Some types of plant cells produce thick "secondary walls" which are impregnated with a complex polymer called lignin.
** Suberin is a wax impermeable to water.

❑ Identify in your pea root sections and label, in Figures 8.4 and 8.5, the following tissues and regions of the root.

The outer covering of the root is the **epidermis**. It is composed of relatively small cells. Unlike the epidermal layer of stems and leaves, the root epidermis is not covered by a waxy cuticle.

?   Why might the epidermal layers of leaves and stems be cutinized while the root epidermis is not?

The root section in Figure 8.4 is from a mature region of the root which does not have root hairs.

Just inside the epidermis is the root **cortex**, composed of a cell type called **parenchyma**. Parenchyma cells are thin-walled, relatively undifferentiated and capable of dividing. Note the many intercellular spaces between the parenchyma cells. Many plants store large amounts of photosynthetic products in the cortex region of their roots. Note the numerous amyloplasts containing starch granules in the parenchyma cells of *Ranunculus*.

The **endodermis** is the innermost layer of cortex cells, which surrounds the vascular cylinder. Unlike the rest of the cortex, the endodermis is composed of compactly arranged cells. Furthermore, the primary cell wall of each young endodermal cell is impregnated with a Casparian strip (Figure 8.3.), a band of waterproof suberin, on the surfaces perpendicular to the root's surface (see Campbell and Reece 2008, p. 746-747).

?   Can you identify the endodermis in your pea root sections? If you can, is it more conspicuous in the younger or the older section?

Water and its dissolved mineral ions can enter the root through the cell walls of epidermal cells and move freely through the loosely packed cortex tissue without entering the protoplasm of the cells. This solution diffuses through the **apoplast**. But, the Casparian strips (Figure 8.3.) of the endodermal cells provide a barrier to the apoplastic movement of the solution into the vascular cylinder. All solutes and water entering or leaving the vascular cylinder through the endodermis must pass through the cytoplasm of the endodermal cells. The results of recent research suggest that the entire endodermis functions as one continuous differentially permeable membrane surrounding the vascular cylinder, and can regulate the passage of different types of solutes, both into and out of the that structure.
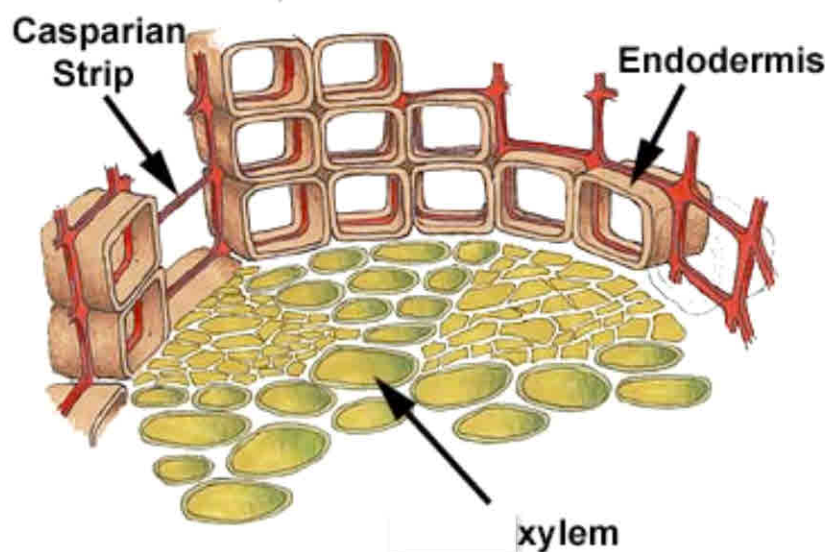
Figure 8.3. Three-dimensional representation of location of Casparian Strips

In the older part of the root, which lacks root hairs and absorbs relatively little water, the endodermis matures and most of its cells become modified so that all their wall surfaces are completely impermeable to water and solutes. A layer of water-impermeable suberin is deposited on all cell wall surfaces of these cells and then secondary cell walls are deposited. Some endodermal cells, however, do not undergo these modifications; they remain thin-walled with only a Casparian strip. These unmodified cells are called passage cells because they permit the movement of substances into and out of the vascular cylinder. The endodermis of the *Ranunculus* root section in Figure 8.5 is mature; most of its cells have heavy cell walls.

Do you see any passage cells?

The **stele** of the root is a vascular cylinder inside the endodermis and occupies the central region of the root (See Figure 8.5). It is composed of the following tissues:

The **pericycle** is a layer of parenchyma cells immediately inside the endodermis. Its cells, which are capable of dividing, give rise to root apical meristems. Branch roots arise from these meristems. (Your lab instructor will project micrographs showing the origin and development of branch roots.)

Plants have two types of **vascular tissue**, each performing a different function. In most dicot angiosperms the **xylem vessels** occupy the central portion of the stele in the root. In *Ranunculus* the xylem region has four or five ridges along its length which extend outward to the pericycle (these ridges appear as "arms" in a cross-section). The conducting "cells" in the xylem of angiosperms are **vessel elements** and **tracheids**, which are elongated, tube-like cells with rather heavy cell walls (stained red in the projected photomicrograph). In their mature, functional form they are devoid of living protoplasm and consist of empty cell walls. Vessel elements abut end to end, forming a conduit called a vessel. Water and dissolved solutes are transported passively through the xylem conduits.

The **phloem** is located, in bundles, between the xylem ridges ("arms"). The phloem conduits, composed of sieve cells abutted end-to-end, (see Figure 8.6) carry organic nutrients into and out of the root. Phloem sieve cells and their adjacent companion cells have thin primary cell walls which are stained green in the projected photomicrograph.

❑   Compare your stained pea root sections to sections of buttercup root shown in Figures 8.4 and 8.5. Attempt to identify all the tissues and regions you labeled on those figures.
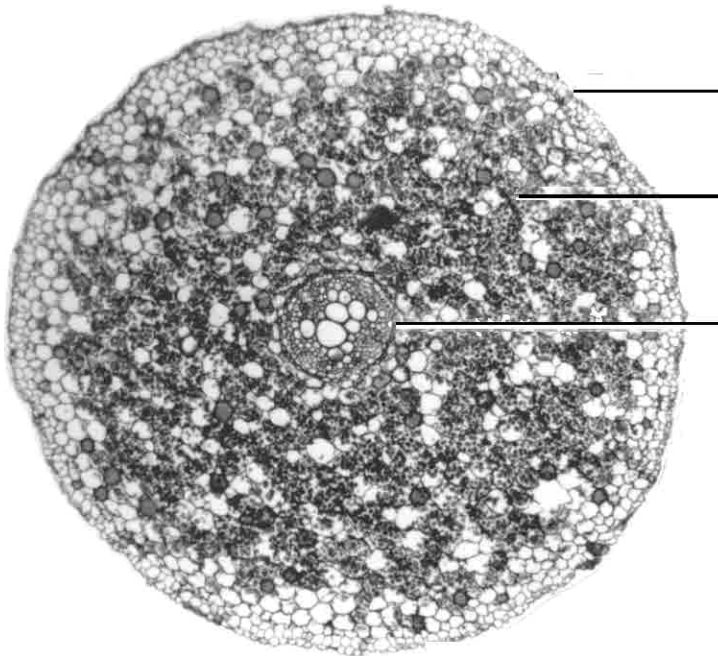
Figure 8.4. Cross-section of a mature root of the eudicot buttercup (*Ranunculus* sp.).
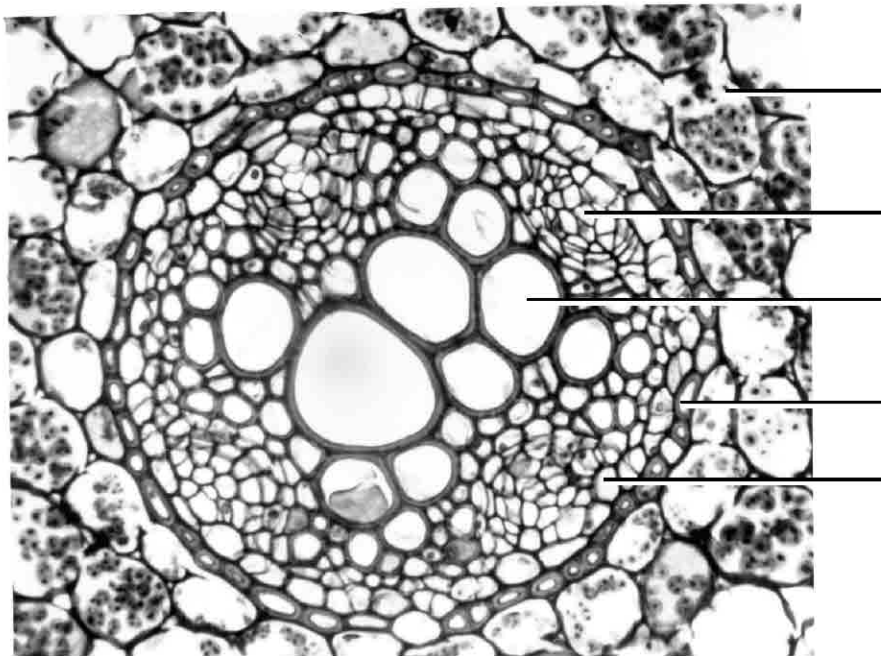
Figure 8.5. Cross-section of a mature root of buttercup (*Ranunculus* sp.) showing the stele in detail.

❑   Observe your collection of root sections and give special attention to xylem vessels. Attempt to find a section in which you can see one or more xylem vessels as continuous open tubes running through the section.

❑   Observe one of your root sections with branch roots. Note how the vascular tissues of the branch root are continuous with the vascular tissues of the primary root.

❑   Observe a projected photomicrograph showing a root cross section with a very young branch root.

?   What region of the stele gives rise to branch roots? _____

Root Pressure

Your lab instructor excised part of a *Setcreacea* sp. shoot at the beginning of the laboratory period. The root system attached to the remainder of the shoot is in water saturated vermiculite.

❑   Observe the shoot stump. You may remove the plastic cover over the plant, but <u>do not move the tub containing the plants</u>. Look for a drop of liquid where the shoot was cut.

The liquid emerging from the stump is sap (water and dissolved solutes) which has been pushed through xylem vessels up the stem by a pressure developed in the roots. This "root pressure" is produced by developing a greater solute concentration, and consequently a more negative water potential, in the xylem sap than in the soil solution. Water diffuses down the ψ gradient from soil to the xylem. As water accumulates in the tubes it can only go upward. The ψ gradient required for root pressure is achieved by the active transport of mineral ions from the soil solution into the cytoplasm of **epidermal** cells (part of the symplast) and the concurrent active transport of mineral ions from the cytoplasm of **pericycle** cells and

other parenchyma cells of the stele (part of the symplast) into their cell walls (part of the apoplast). The ions then diffuse into the walls and lumens of xylem vessels and tracheid conduits (part of the apoplast). The ions diffuse, perhaps with the aid of cytoplasmic streaming, through the symplast from the epidermis into the stele. Figure 8.6 and Figure 8.7 summarizes this information.

©http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/X/Xylem.html
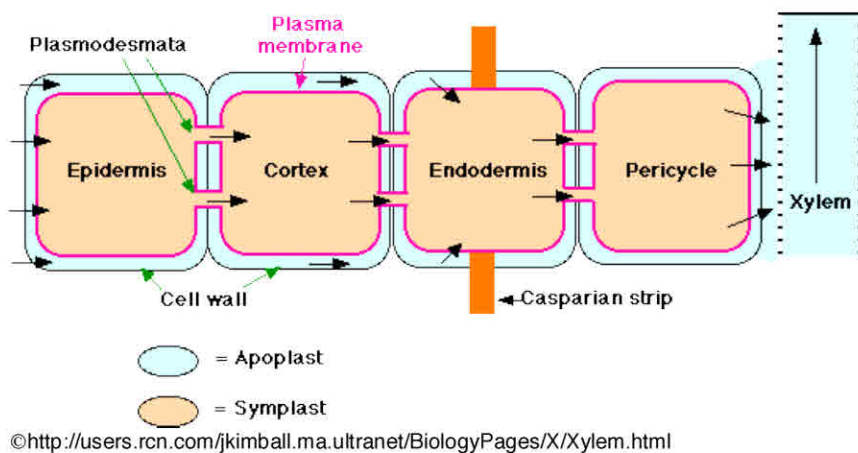
Figure 8.6. Schematic diagram representing apoplastic and symplastic movement of water.

SOIL		EPIDERMIS		CORTEX				STELE			
				PARENCHYMA		ENDODERMIS		PERICYCLE & OTHER PARENCHYMA CELLS			XYLEM TUBES
MINERAL IONS	→ AT	symplast	→	symplast	→	symplast	→	Symplast	→ AT	apoplast	→ apoplast
				Ions move through symplast by diffusion (maybe facilitated by cytoplasmic streaming)							
WATER	→	apoplast	→	apoplast	→	symplast	→	apoplast	→		apoplast
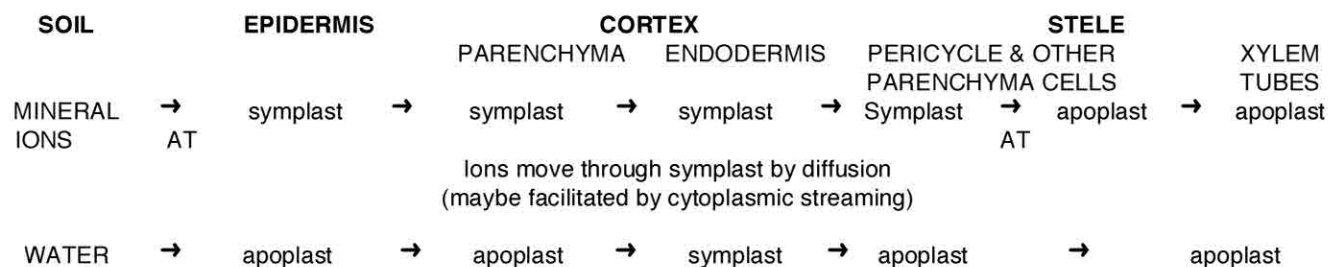
Figure 8.7. The pathways of mineral ions and water molecules from the soil into the root stele of a vascular plant. Some water molecules follow the pathway of mineral ions. AT signifies active transport through cell membranes.

Typically root pressure can only force water up about ten meters, it is also energetically expensive to generate, and obviously during conditions favoring transpiration the root pressure mechanism is not used since there is a tension in the xylem conduits at those times. Thus most plants will use the transpiration-cohesion-tension mechanism when they can, and only resort to the root pressure mechanism when they must. In either case the movement of ions from the cytoplasm of the pericycle cells into the apoplast of the stele is the means used by vascular plants to get the essential minerals needed by the shoots into the xylem sap for movement by either the root pressure or transpirational mechanisms to the shoots.

Anatomy of the Stem

❑   Select and focus on your best sections of *Impatiens* sp. stem. Compare your stem sections to the section of *Helianthus* sp. (sunflower) shown in Figure 8.6.

?   What part of your *Impatiens* sp. sections are stained blue green? What does this indicate?

As you examine cross-sections of the stem, consider its major functions and relate them to the structures you observe. The stem displays the leaves so that a large surface area is exposed to light for photosynthesis. Leaves are separated along the stem axis, and if the stem is upright, structural support of the stem is required; consequently, the stem has more mechanical or supportive tissue than does the root. Furthermore, the stem provides the vascular connections between the roots and the leaves. The vascular system (**stele**) is not organized into a central cylinder as in the root, but instead it is usually organized into distinct bundles nearer the surface of the stem. One or more of the vascular bundles branches off the stem to form a vascular connection with a leaf.

The vascular bundles in dicot stems are usually arranged in a ring inside the periphery of the stem (see Figure 8.8). Most dicot species have distinct vascular bundles separated from one another by ground tissue, but some species have a continuous ring of vascular tissue. The region of ground tissue between the epidermis and the vascular tissue is the **cortex**, and the region of ground tissue inside the vascular tissue is called the **pith** of the stem. *Helianthus* sp. and many other dicots have a bundle of **sclerenchyma** fibers immediately outside each vascular bundle. These fibers are elongate, heavy-walled cells which collectively form a supporting tissue adjacent to vascular bundles. *Impatiens* sp. does not produce large bundles of fibers next to the vascular bundles.

In angiosperms there is a transition in the vascular tissue from the more compact and central arrangement in the root (see Figure 8.4) to a more open and peripheral arrangement in the stem (see Figure 8.8a). In this transition region the endodermis, characteristic of the root, does not continue into the stem. The individual vascular bundles of the stem do not have endodermal tubes around them.

Notice that the xylem is to the inside and the phloem is to the outside in the vascular bundle. This arrangement of vascular tissues in the bundle is typical of many species, but there are several exceptions; for example, some have phloem on both the inside and the outside of the xylem.

A.
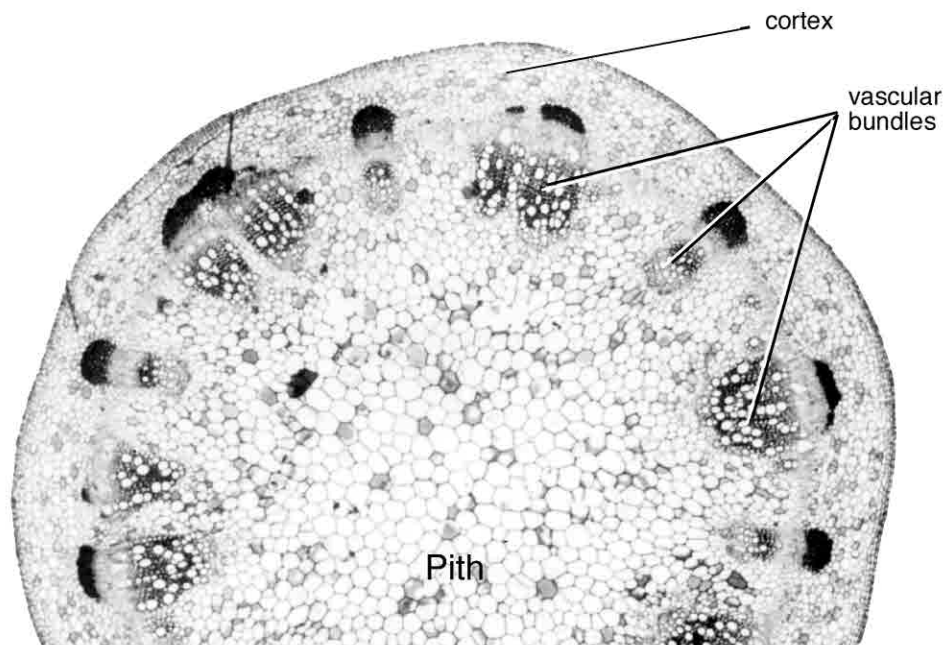
Figure 8.8 continued on the next page.

B.
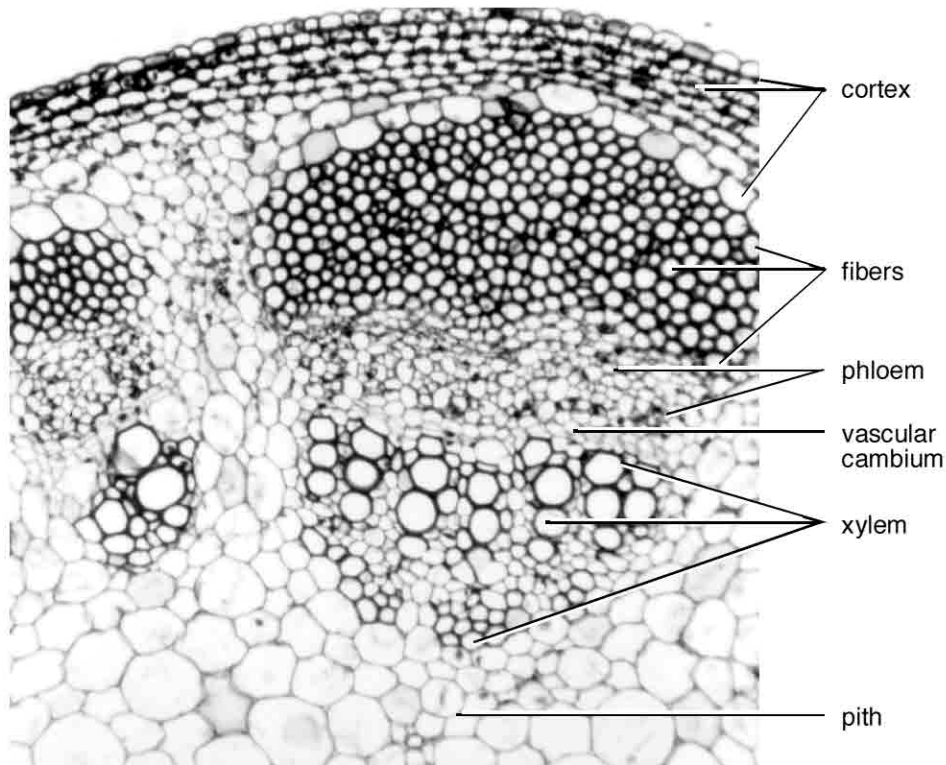
Figure 8.8. Cross-sections of the stem of sunflower (*Helianthus* sp.), an herbaceous dicot. A. low power magnification showing the ring of vascular bundles. Notice the variation in bundle size. B. higher power magnification showing two vascular bundles and adjacent tissues. The tissues in B are younger than those in A. In B a vascular cambium is present between the xylem and phloem in each vascular bundle, but it has not formed between bundles as it has in A.

❑ Observe one good section of *Impatiens* stem. Look for a distinct ring, composed of small cells, that passes through each vascular bundle and between the vascular bundles. See Figure 8.8A.

This ring consists of the **vascular cambium** which is a meristem, and the cells produced by it. Actually, in three dimensions, the vascular cambium is a tube. Cells produced on the outside of the vascular cambium become phloem cells. Cells produced on the inside become xylem (wood). If the ring is relatively thick, its innermost cells may have matured enough to have lignified secondary cell walls which are stained blue-green.

Although *Impatiens* is regarded as an herbaceous plant, it does make a small amount of wood in the older parts of its stems and roots. Wood is the xylem produced by the vascular cambium. Many types of herbaceous dicots make wood in the older regions of their stems, but the tissue is lost along with the rest of the shoot system when it dies at the end of the growing season.

The meristems and specialized tissues of woody perennial dicots are not covered in this laboratory.

Structure of the Leaf

Leaf External Morphology

❑   Obtain one of the leaves indicated by your lab instructor and study its external morphology.

The major part of the photosynthetic tissue is distributed in a flattened structure called the leaf **blade** or **lamina**. In most dicots the blade is attached to the stem by a stalk, the **petiole**, which is stem-like in appearance. A portion of the vascular tissue in the stem branches off and continues into the leaf; in the leaf blade it branches repeatedly, forming the numerous conspicuous veins. Branching of the vascular system in the blade is so extensive that each photosynthetic cell is no more than a few cells away from a vein (see Figure 8.9).

Figure 8.9. Surface view of a portion of a cleared leaf of the dicot *Liriodendron tulipifera* ("tulip tree") showing the branching of the vascular system. This venation pattern is called "netted."
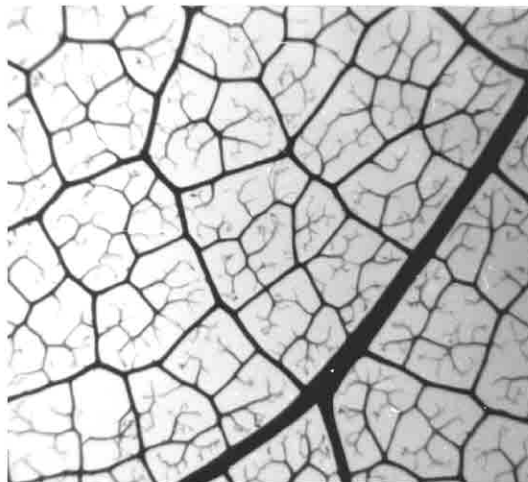
Leaf Anatomy of Privet (*Ligustrum* sp.)

❑   Obtain a prepared slide labeled "Ligustrum leaf," and observe the leaf cross section with a compound microscope.

Your lab instructor will project photomicrographs of similar dicot leaf sections and help you identify tissues and structures.

❑   Focus on the midvein in your leaf section and observe it at 400X magnification. Notice photosynthetic cells with chloroplasts, which look like granules, in them on the sides of the large vascular bundle. Also note nonphotosynthetic cells with rather thick cell walls above and below the vascular bundle.

?   What is the most likely function of the thick-walled cells on the upper and lower parts of the midvein?

❏   Continue to use 400X magnification and move the slide so you can view a portion of the leaf on one side of the midvein. Use Figure 8.10 and the following text to identify these tissues, regions and structures:

epidermis, guard cells and stomata
spongy mesophyll, palisade mesophyll
vascular bundles, bundle sheath

upper epidermis
palisade mesophyll
vascular bundle
spongy mesophyll
bundle sheath cells
lower epidermis
intercellular space   pair of guard cells
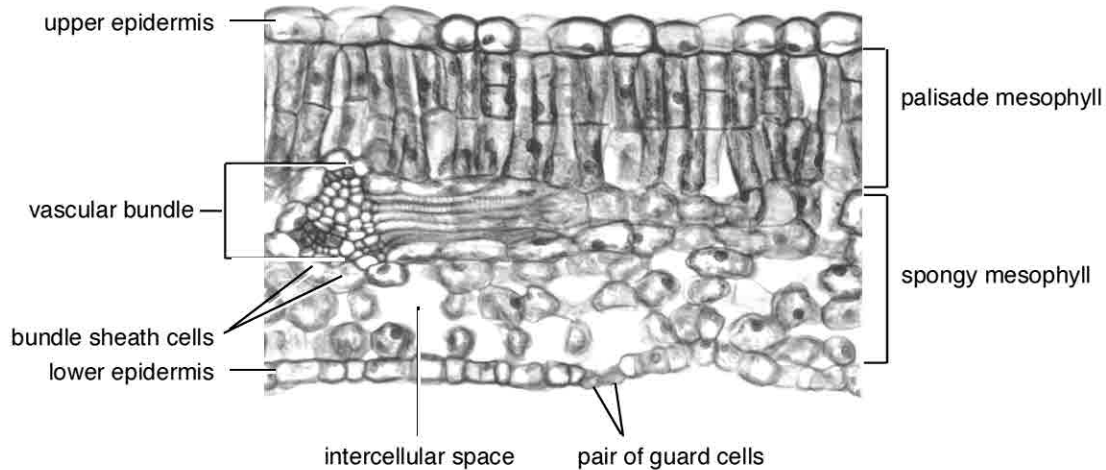
Figure 8.10. Cross-section of a privet (*Ligustrum*) leaf, x430. Privet is a dicot and photosynthetically a C$_3$ plant. The vascular bundle runs obliquely to the plane of the leaf section; therefore, both transverse and longitudinal views of the bundle are shown.

The **epidermis**, the upper and lower surface tissue of the leaf, is composed mainly of cells which fit together tightly. Pores in the epidermis, called **stomata** (sing.; stoma) are formed by pairs of specialized epidermal cells called **guard cells**. Stomata allow exchange of gases between the leaf's interior and the outside environment. Note an intercellular space next to each stoma inside the leaf. Generally the guard cells are the only photosynthetic cells of the epidermis; other epidermal cells lack chloroplasts. Also, guard cells generally are smaller than other epidermal cells.

?   Which epidermis (upper or lower) of a *Ligustrum* leaf has the greater number of stomata?

Small multicellular structures projecting outward from the epidermis are glands which secrete various substances.

The photosynthetic region of the leaf is the **mesophyll** between the two epidermal layers. Leaves of some species have only a **spongy mesophyll** which consists of loosely arranged cells having much space between them. Leaves of many species also have a **palisade mesophyll** composed of cylindrical cells more compactly arranged than those of the spongy mesophyll. Mesophyll cells are specialized parenchyma cells with chloroplasts. The intercellular space of the mesophyll is normally filled with gases including nitrogen, oxygen, carbon dioxide and water vapor.

In your leaf cross section you can see a small part of the leaf's vascular network in cross-, longitudinal- and oblique sections of **vascular bundles** (also called veins).

❏   If you do not understand why you see this variety of vein sections, draw a line perpendicular to the largest vein in Figure 8.9 and observe how the smaller veins intersect with the line.

?   Vascular bundles in the leaf are continuous with one or more bundles in the stem. Xylem is generally interior to phloem in the vascular bundle of the stem. If this same arrangement of xylem and phloem is maintained in the leaf, is xylem above or below phloem in a leaf vein?

Each vascular bundle is surrounded by a layer of tightly packed cells called a **bundle sheath**. Generally bundle sheaths extend to the ends of the veins so that no part of the vascular tissue is exposed directly to gases in the intercellular spaces. Furthermore, any substance which moves between the vascular tissue and the mesophyll must pass through the bundle sheath. Thus the bundle sheaths of the leaf perform a function similar to that of the endodermis in the root; however, bundle sheath cells do not have Casparian strips in their walls.

?   Are the bundle sheath cells of *Ligustrum* photosynthetic? What anatomical evidence do you have for your answer?

The Epidermal Surface of the Leaf

Certain studies require information regarding the distribution and density of stomata in plant epidermal layers (Table 8.2). Because guard cells are extremely small, the resolving power and magnification of a compound microscope are required to view them. The leaves of some plants are thin enough to transmit a sufficient amount of light to enable one using a compound microscope to see guard cells in an intact epidermis. Unfortunately this technique is not practical with thick plant structures because the tissues beneath the epidermis absorb too much light. Other approaches used to view the fine features of the epidermis are to peel it from the structure and view the excised tissue, or make and view a replica of the epidermis.

❏   Procedure for preparing leaf pieces for microscopic study of intact epidermis.

1.   Obtain a healthy purple leaf from the *Setcreacea* shoot which was cut off for the root pressure demonstration. (One leaf will serve several students.)

2.   Use a razor blade and a cutting board to cut a 1 cm x 1 cm (approximately) piece of the leaf.

3.   Dip your finger in water and spread it on a microscope slide, then place the leaf piece, bottom side up, on the microscope slide. (The water attaches the leaf to the glass.)

4.   Observe the leaf piece with a compound microscope. First use the 4 X objective to focus on the epidermis, then use higher power objectives. The highest light intensity is required.

❏   Look for small, pale green or colorless rectangles among the many purple cells of the epidermis. Each rectangle contains a stoma.

Each stoma is in a "box" consisting of six cells:
" a pair of guard cells
" a subsidiary cell beside each guard cell (see Figure 8.11)
" a cell on each end of the guard and subsidiary cells.

Figure 8.11. Surface view of a ***Tradescantia*** **leaf epidermis showing stomata, x450. The guard cells and the subsidiary cells are smaller than other epidermal cells in this and many other species. In some species, however, subsidiary cells are indistinguishable from the other epidermal cells.**

guard cell

subsidiary cell
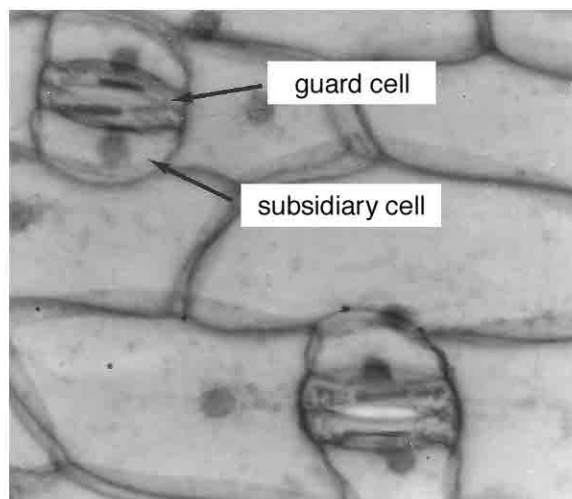
Stomata are not present in the epidermis on the top side of *Setcreacea* leaves. Most of the epidermal cells on the bottom side of the leaves are not photosynthetic; they contain anthocyanin, a purple pigment, in their vacuoles. The guard cells of *Setcreacea* are relatively large. They are easily distinguished at 100X magnification.

❑   Count the number of stomata you observe in a single <u>100X</u> field of view.

Number of stomata in a 100X field of view of *Setcreacea* leaf epidermis: _____

❑   Obtain a 1 cm x 1 cm (approximately) piece of a healthy *Impatiens* leaf without major veins and adhere it, bottom side up, with water beside the *Setcreacea* leaf piece on the microscope slide.

❑   Observe the *Impatiens* leaf piece with the microscope, again beginning with the 4X objective and then with higher power objectives. Look for stomata.

The guard cells of *Impatiens* are much smaller than other epidermal cells; they are too small to be seen with the 4X objective and can barely be distinguished with the 10X objective.

❑   Use the 40X objective and identify stomata.

A guard cell pair looks like a boat if the pore is closed or nearly closed, or like an oblong donut if the pore is widely open.

❑   Count the number of stomata you observe in a single <u>400X</u> field of view.

Number of stomata in a 400X field of view of *Impatiens* leaf epidermis: _____

❑   Record your stomata counts in the table on the transparency.

Table 8.2 Stomatal density on leaves of various plants (adapted from Sutcliff 1968).

Species	Mean number of stomata per cm^3	
	Upper Epidermis	Lower Epidermis
Bean *(Phaseolus vulgaris)*	4000	28100
Geranium *(Pelargonium sp.)*	1900	5900
Ivy *(Hedera helix)*	0	15800
Maize *(Zea mays)*	5200	6800
Oat *(Avena sativa)*	2500	2300
Sunflower *(Helianthus annus)*	8500	15600
Tomato *(Lycopersicon esculentum)*	1200	13000
Wandering Jew *(Zebrina pendula)*	0	1400
Wheat *(Triticum sativum)*	3300	1400

DYE TRANSPORT IN AN ANGIOSPERM SHOOT–A DEMONSTRATION

Shortly before your laboratory period began, the larger leaves were removed from a flowering shoot of *Impatiens*, then the shoot, itself, was cut from the plant. A small piece of the basal end of the excised stem was cut off under water to ensure that air did not enter the xylem conduits of the excised shoot. The cut shoot base was kept in water until your lab instructor transferred it to the blue dye in a test tube. The shoot has been illuminated in the breeze from a fan since the beginning of the lab period.

❑ Observe the excised shoot with its base in the dye.

? Has the blue dye reached the flower petals?

? Can you see columns of blue dye in the stem? You may need to hold the shoot in front of a light to see this.

POST-LAB WEB ASSIGNMENT

To test your understanding of this laboratory, complete the associated web activities located in the lab section of the course web site.

URL = http://biog-101-104.bio.cornell.edu/BioG101_104/tutorials/botany.html

You will encounter questions similar to these on major quizzes and the practical examination.

REFERENCES AND SUGGESTED READINGS

Campbell, NA.; Reece, JB. Biology. 8th ed. San Francisco, CA: Pearson-Benjamin/Cummings ; 2008.

Raven PH, Evert RF, Eichhorn SE. 1999. Biology of plants. 6th ed. New York: WH Freeman and Co./Worth Publishers.

Sutcliff J. 1968. Plants and water. New York: St. Martin's Press.

ANGIOSPERM STRUCTURE AND FUCTION WORKSHEET

Name_____   **Section**_____

NOTE: The flower petals of *Impatiens* are structurally similar to leaves. They have vascular bundles, a nonphotosynthetic mesophyll with much intercellular space and stomata in the epidermis.

1.   a.   Water always moves down a water potential gradient. In the dye solution-*Impatiens* shoot-air system you observed, which of the three items has the most negative water potential?

           dye solution   *Impatiens* shoot   air

        b.   What determines the ψ of that item?

2.   What is the main evaporative surface for water in the leaf?

3.   Why were the larger leaves removed from the *Impatiens* shoot used in the dye transport demonstration?

4.   The *Impatiens* shoot was placed in a breeze to increase the rate of transpiration by the leaves/petals. How does air movement over a leaf surface increase the evaporative loss of water from the leaf?

5.   a.   What is the force that moved the dye solution up through the xylem conduits of the *Impatiens* shoot?

        b.   What produced that force?

6.   A requirement for the movement of water up through the xylem conduits by the transpiration–cohesion–tension mechanism is continuous columns of water in the conduits. How are those continuous water columns maintained (i.e., what prevents the columns from breaking)?

7.   a.   In the dye solution-*Impatiens* shoot-air system you observed, was the dye solution moving in the apoplast or the symplast of the shoot? Explain your answer.

      b.   In what tissue of the leaf/petal were the dye molecules left as water evaporated? (Refer to Figure 8.10)

8.   If an entire *Impatiens* plant with its attached root system immersed in the dye solution had been used in the demonstration, what part of the root system might have interfered with the movement of dye molecules through the plant? Explain your answer.

9.   The transport of a solution in the xylem conduits from the root system into the shoot can occur by the development of root pressure or by the evaporation-cohesion-tension mechanism. Either mechanism causes water to move from the soil into the xylem conduits by producing a water potential gradient between the soil solution and the sap in the xylem conduits of the roots.

      Recall that the water potential of a solution is the algebraic sum of the solute potential (ψ_s) of the solution and the pressure (ψ_p) on the solution. In the blank following the name of each mechanism write the component (ψ_s or ψ_p) which has the greater effect in producing the negative water potential of the xylem sap used by that mechanism.

      Root pressure mechanism: _____

      Transpiration-cohesion-tension mechanism: _____

10.   **Estimation of Stomatal Density in an Epidermis**

Record the <u>mean</u> values for stomata counts in your lab section.

Mean no. of stomata in *Setcreacea* leaf epidermis in a 100X viewing field: _____

Mean no. of stomata in *Impatiens* leaf epidermis in a 400X viewing field: _____

The viewing field diameter is 1.8 mm at 100X magnification and 0.45 mm at 400X magnification on your compound microscope. Calculate the area of the viewing field at these two magnifications.

Area of 100X viewing field: _____   Area of 400X viewing field: _____

Calculate the stomatal density of the leaf's lower epidermis for the two plants.

Stomatal density of *Setcreacea* leaf epidermis

a. _____ per mm^2 b. _____ per cm^2

Stomatal density of *Impatiens* leaf epidermis

a. _____ per mm^2 b. _____ per cm^2

CHAPTER 9 – BIOMECHANICAL ANALYSIS OF VERTEBRATE SKELETAL SYSTEMS

LABORATORY SYNOPSIS

In this two-day laboratory, you will investigate certain mechanical considerations in the evolution of vertebrate skeletal systems. You will learn elementary biomechanical principles using a simplified kinematic model of a human foot/ankle system. Once you have become familiar with the basic concepts of vertebrates as biological machines, the second day will be spent analyzing the articulated skeletons of a few representative vertebrates in an effort to 1) correlate biomechanical structure with known locomotory and feeding function and 2) aid your understanding of the anatomical basis used to establish the evolutionary relationships among vertebrate groups. You will see how natural selection and the laws of physics have combined to produce the movements exhibited by vertebrates and shaped vertebrate anatomy.

LEARNING OBJECTIVES

Upon completion of this laboratory, you should

1. understand the physical concepts relating to levers: moment arm, in-force and out-force, torque, mechanical advantage, and input and output speed.

2. know how the ratio of output moment arm to input moment arm is related to the mechanical advantage of a lever system.

3. know how the ratio of output moment arm to input moment arm is related to the speed ratio of a lever system.

4. be able to apply these concepts when analyzing skeletal systems from a structure-function viewpoint.

5. know the meanings of the terms: dorsal, ventral, anterior and posterior with respect to an animal's body.

6. understand why muscle groups are arranged in particular anatomical configurations in order to produce the movements characteristic of vertebrate animals.

7. understand the evolutionary relationships among the major vertebrate groups based on similarities and differences in their skeletal structure.

8. understand basic features of the representative skeletons viewed.

READING ASSIGNMENTS (should be done <u>before</u> lab period.)

> **Day 1**
> In Campbell and Reece (2008):
> > Chapter 34: VERTEBRATES   p. 698-735
> > Chapter 50: SENSORY AND MOTOR MECHANISMS   p. 1087-1119
>
> In the lab manual   p. 167-175
>
> **Day 2**
> In the lab manual
> > ANALYSIS OF ARTICULATED VERTEBRATE SKELETONS   p. 183-196
> > How Animals Run   p. 201-208

SPECIAL NOTE: Bring a calculator to this laboratory.

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

1.   What are the main functions of tendons, ligaments, and cartilage?

2.   What is a lever?

3.   What is meant by in-force and out-force?

4.   What is torque and how does it differ from force?

5.   What is the relationship between in-torque and out-torque?

6.   To see if you understand the Law of the Lever, let's try a few problems.

    (a)   If F_i = 50 N, its moment arm (S_i) = 2.0 m, and the other moment arm (S_o) = 4.0 m, what is F_o? Show all appropriate formulas and calculations. Disregard the weights of the lever lengths in the moment arms.

    (b)   An arm is pushing against an object that has a resistance force (F_r) of 200 N, S_o = 0.30 m, and S_i = 0.15 m. What is the minimum force required to move the object? Again, show all appropriate formulas and calculations, and disregard the weights of the moment arms.

7.   Why can muscle/skeletal systems be considered lever systems?

8.   Give the definition of each of the following terms with respect to an animal's body: dorsal, ventral, anterior, posterior.

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Revised June 2010
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INTRODUCTION

Members of the subphylum Vertebrata are characterized by the presence of an endoskeleton which includes a backbone composed of a series of vertebrae. The vertebrae are arranged into a vertebral column, which makes up the axial skeleton of the animal. The appendages (e.g., fins, legs, wings) and components that support the appendages comprise the **appendicular** skeleton. Throughout vertebrate evolution, both the axial and appendicular skeletons have undergone specific modifications in form which have enabled animals to meet the functional requirements of animals living in water, on land, or in the air. Nevertheless, virtually all of the major skeletal elements can still be recognized in every vertebrate species. Systematists include similarities and differences in skeletal modifications in the criteria they use to determine evolutionary relationships among various vertebrates.

In this laboratory you will study in detail the articulated skeletons of representatives from three classes of vertebrates -- Amphibia, Aves and Mammalia. These skeletons show the results of major evolutionary modifications of skeletons for various methods of feeding and locomotion. Adaptations for flight are shown in the skeletons of a bird and a bat. The frog skeleton shows adaptations for jumping and swimming. The mammalian skeletons show adaptations for brachiating (swinging by the arms) and standing upright in the human; high speed running in the cat and horse; and burrowing in the mole.

The following is a brief account of the major changes in skeletal structure which occurred in the evolutionary sequences that gave rise to the amphibians, reptiles, birds and mammals. For a more detailed discussion on the characteristics and evolution of these classes (see Figure 34.2, p. 699 in Campbell and Reece 2008).

Members of the class Amphibia were probably the first vertebrates to spend much of their time out of the water. Amphibians are descendants of a particular group of bony fish known as the lobe-finned fishes. To adapt to the inherent differences between life on land and in water, the amphibians had to undergo several significant skeletal modifications. These included the changing of paired fins into terrestrial legs, and the development of flattened projections within the pectoral (shoulder) and pelvic (hip) regions to support the musculature necessary to move these limbs against the pull of gravity. In addition, the amphibian axial skeleton developed extensions from the vertebrae in the form of a primitive rib cage, thus providing support for internal organs which had previously been suspended by the buoyancy of the ancestral aquatic medium. However, for reasons having to do primarily with reproduction, the amphibians never became completely independent of an aquatic habitat.

Certain types of ancient amphibians gave rise to another group of vertebrates now known as the class Reptilia. These animals represent the first truly terrestrial vertebrates, having overcome the problems of reproduction outside an aquatic habitat by development of the desiccation-resistant amniotic egg. Although some reptiles have secondarily lost their locomotory appendages (e.g. snakes), the basic skeletal modifications that are observed in the transition from fish to amphibians have in general been maintained and enhanced by the reptiles. Reptilian legs are usually larger and stronger than those of amphibians, and in many cases are attached lower on the sides of the body. This positioning permits the animal to raise its body off the ground, thus making locomotion more efficient. As a result the range of motion possible with reptilian limbs generally exceeds that of amphibians.

Early in the evolution of reptiles, the members of this class separated into several different lineages, four of which gave rise to major groups of existing species. One evolutionary line gave rise to the **turtles**. Another line produced the **snakes** and **lizards**. A third group evolved more-or-less directly into the ancestors of the **mammals**. A fourth line produced **crocodilians** and **dinosaurs**. All the dinosaurs are extinct, but one group of them evolved into **birds**.

As a class, the birds have successfully exploited the aerial environment better than any other group of animals (with the exception of insects), and as might be expected, their skeletal plan shows major modifications for life in the air. Most of these modifications center around the need for reduced weight and increased stability, both prerequisites for flight. Structures such as teeth and solid bones, which are found

in almost all other vertebrates, have been reduced through evolution in order to conserve weight. Portions of the vertebral column are fused to provide a more rigid backbone. Parts of the appendicular skeleton are enlarged to hold the large flight muscles and strengthened to withstand the stresses produced by those muscles.

Among the modern vertebrates, members of the class Mammalia have the greatest range of sizes. The giant blue whale measures over 30 meters in length and weighs 10^6 kg; the tiny tree shrew is about 5 cm long and weighs less than 30 g. The truly terrestrial mammals have numerous skeletal adaptations which have enabled them to be extremely successful on land. Their limbs are directly under the body to maximize length of stride and increase stability. The limb bones of many mammals are large and strong to support the stress of substantial body size and rapid locomotion. The pectoral and pelvic regions are enlarged to provide attachment sites for the musculature necessary to move these larger limbs. The rib cage is extensive to support the enlarged visceral mass necessary to sustain large body size. These characteristics, however, are not unique to the terrestrial mammals; the terrestrial dinosaurs also possessed them.

It should be clear that a major factor contributing to the success of vertebrates has been the evolution of a highly adaptable endoskeletal system which is organized as a series of jointed levers operated by specific muscles. In fact, in this laboratory we shall regard vertebrates as machines consisting of numerous lever systems composed of bones and muscles held together by ligaments and tendons. In some cases a bone-muscle lever system may function alone, but usually it works in concert with other connected lever systems to accomplish movement by the organism. Because the muscles and bones of vertebrates are primarily arranged in systems of simple levers, we can analyze the mechanical properties of their skeletons and thereby understand the characteristics and potentials of movement as a whole. The study of animal form and how it is used generally falls under the heading of **functional morphology**, a branch of biology that considers how the structure of an organism determines the function of its various body parts as they relate to feeding, locomotion, and other aspects of life.

An article by Hildebrand (1974) entitled "How Animals Run" is included at the end of this chapter. Hildebrand describes skeletal adaptations, primarily in the legs and feet, of cursorial mammals such as cats and horses which enable them to run fast and easily.

APPLYING THE LAWS OF MOTION TO ANIMAL LOCOMOTION

For an animal to move by its own efforts, it must elicit a force (a push) <u>from</u> the external environment. This is accomplished by the contraction of the animal's muscles to move body parts which push against some part of the organism's external environment. Concurrently, the environmental component exerts an equal but <u>opposite</u> force against the animal. As the animal applies force on its environmental component, the component, or the animal, or both the animal and the component, may be moved. The animal's body moves because the environmental component resists the movement of the animal's body part.

How do these principles apply generally to the action of muscle and bone in vertebrates? To answer this question we must begin by examining the structure of vertebrate skeletal components in some detail.

MUSCLE/SKELETAL CONNECTIONS

A typical skeletal muscle has three basic areas (Figure 9.1). The muscle's ends are usually attached to some skeletal part, such as bone or cartilage, via **tendons**. The muscle's **origin** is attached to a stationary bone and is therefore relatively non-movable (with reference to the joint under consideration),

whereas the **insertion** is attached to a more movable bone. The enlarged belly, or center section of the muscle, contains many fibers which contract and pull on the bone at the muscle's insertion. This bone then moves about the joint located between the stationary and movable bones. In Figure 9.1 the joint of interest is the elbow, located at the junction of the humerus (stationary bone) and the radius/ulna complex (movable bones). Also pictured are the **ligaments**, which connect one bone to another at points of articulation, thus providing additional support to the skeleton. The **cartilage** cushions on the articulating surfaces of bones are not shown.
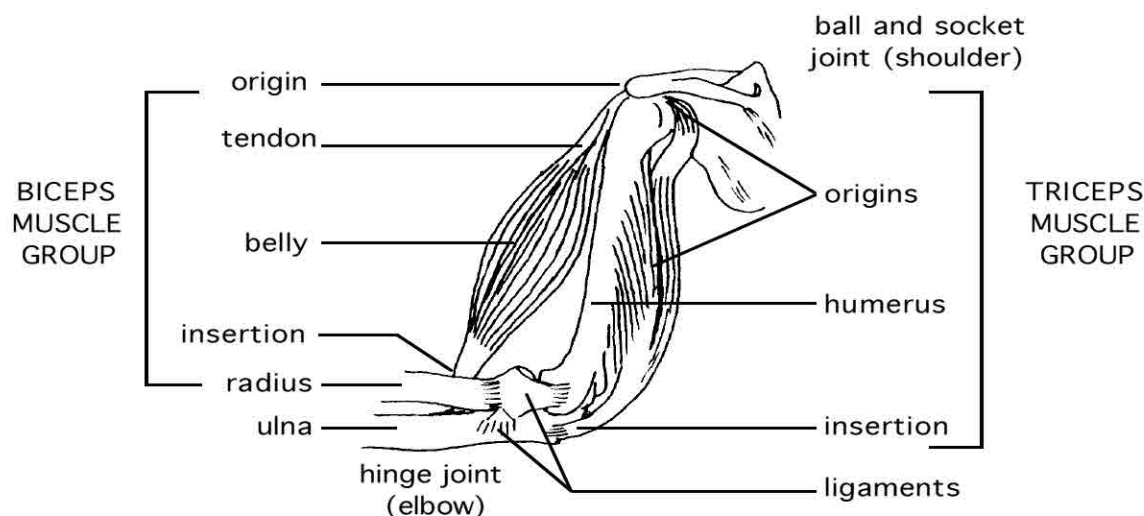
Figure 9.1. Diagram of the muscle/skeletal connections of the human upper arm showing the typical parts of a muscle.

Contraction (shortening) of muscles cause bones to move about their joints. Since force is generated only when a muscle contracts (as opposed to when it relaxes or stretches), a muscle can only pull (never push) the bone to which it is attached. To move the same bone in the opposite direction, a second muscle or muscle group must contract and pull the bone back. Such pairs of muscles or muscle groups are known as **antagonistic** muscles. An example of antagonistic muscles are those involved in the movement of your forearm as it pivots about the elbow (refer to Figure 9.1). One muscle group (the biceps muscle) causes the forearm to flex, or be pulled closer to your body, while an antagonistic group (the triceps muscle) causes the forearm to extend away from your body. Antagonistic muscle groups such as these are called **flexors** and **extensors**. Convince yourself of the antagonistic nature of these muscle groups by observing the tension in your biceps and triceps muscles as you alternately flex and then extend your lower arm. You will probably find this phenomenon easier to observe if you put a load on the muscles by lifting something in your hand and pushing down on something with your hand.

SIMPLE LEVERS

All bone/muscle systems are machines if we define a machine as a system of connected rigid bodies which transmits force from one place to another. During force transmission, the machine usually changes the magnitude of the original force. It is useful to designate any force applied to a machine as an **in-force** (F_i), and any force derived from a machine as an **out-force** (F_o). In the body, in-forces are applied by the pull of tendons or ligaments resulting from muscle contraction. These forces are then transmitted through the bone, and useful out-forces are ultimately derived elsewhere within the body (e.g. teeth, feet, or digits). For the most part, we will consider only simple machines having one in-force and one out-force.

Most feeding and locomotory systems of the body transmit forces by means of **levers**. A lever is a rigid structure (e.g., an iron bar, a wooden beam, or a bone) that transmits forces by pivoting on a support known as the **fulcrum**. Many everyday tools use levers; common examples are crowbars, wrenches, and pliers. Even some of our favorite childhood toys are based on the principles of leverage. Consider, for example, the seesaw. In order to obtain a balanced seesaw, the two individuals involved must adjust their positions relative to the fulcrum in accordance with their body weights. Let's consider why this is true.

In Figure 9.2, F_A and F_B are the in-forces exerted on the lever (seesaw) by the weight of individuals A and B, respectively. Each force has a **moment arm** which is the <u>perpendicular distance from the axis of rotation to the line of action of the force</u>. The distance separating A from the fulcrum is A's moment arm, abbreviated S_A. B's distance from the fulcrum is the moment arm S_B. F_A causes the lever to turn about the fulcrum in a counterclockwise direction, and F_B causes it to turn in a clockwise direction. The turning effect that a force exerts on a lever system is called **torque** (T), and equals <u>the magnitude of the force times the length of the moment arm</u>. Thus, individual A creates a counterclockwise torque on the lever which is equal to $F_A S_A$. B's torque is clockwise in direction and equal to $F_B S_B$. In general, if $F_A S_A >$ $F_B S_B$ the lever rotates in the direction of F_A (i.e., counterclockwise). When $F_A S_A < F_B S_B$, the lever rotates in the direction of F_B (i.e., clockwise). A lever is said to be in a state of <u>equilibrium</u> when the algebraic sum of all the torques acting upon it equals zero. That is, a lever is in equilibrium when the sum of all the torques that produce counterclockwise rotation equals the sum of all the torques that produce clockwise rotation.

In the specific case of our seesaw example below, the lever is at equilibrium (no motion of the lever) when: $\mathbf{F_A S_A = F_B S_B}$
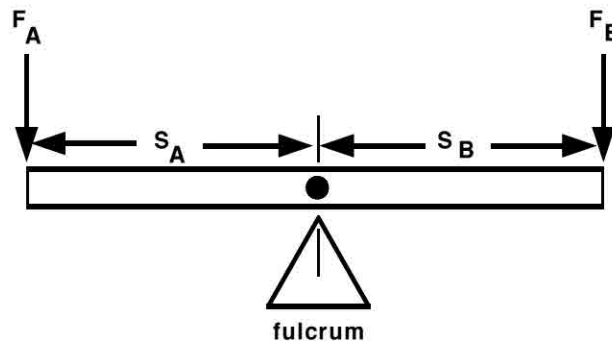
Figure 9.2. A seesaw as an example of a simple lever system.

Let us put some numbers with this example. Suppose two people are balanced (no motion) on the seesaw in Figure 9.2. Person A weighs 90 pounds (= F_A, force in a counterclockwise direction) and sits 4 feet from the fulcrum (=S_A). Person B sits 4 feet from the fulcrum. What force (or weight) must person B exert if the seesaw is to remain balanced (at equilibrium)?

$$\textbf{If} \qquad F_A \textbf{ x } S_A = F_B \textbf{ x } S_B$$

$$\textbf{then} \qquad 90 \text{ lb } \textbf{x } 4 \text{ ft} = F_B \textbf{ x } 4 \text{ ft}$$

$$\frac{360 \text{ lb-ft}}{4 \text{ ft}} = F_B$$

$$F_B = 90 \text{ lb}$$

What is the torque exerted by A (counterclockwise torque)? Ans. $F_A S_A$ = 360 lb-ft.

What is the torque of B (clockwise torque)? _____

Therefore, in order to obtain a balanced seesaw with no movement (i.e., the equilibrium condition), the two participants must adjust their positions from the fulcrum so that the clockwise and counterclockwise torques which they produce are equal. If both individuals are about the same weight, then they should be about equal distances from the pivot point in order to achieve a balanced lever system. Put another way, since their weight forces are equal ($F_A = F_B$), then their moment arms must also be equal ($S_A = S_B$) if the clockwise and counterclockwise torques are to be equal ($F_A S_A = F_B S_B$).

If, on the other hand, individual A is heavier than individual B ($F_A > F_B$), then A's moment arm must be proportionately shorter than B's if $F_A S_A$ is still to equal $F_B S_B$. In the above example suppose individual A weighs 180 pounds. Where must A sit in order to balance the 90-pound individual B who sits 4 feet from the fulcrum?

$$\textbf{If} \qquad F_A \ \textbf{x} \ S_A \ = \ F_B \ \textbf{x} \ S_B$$

$$\textbf{then} \qquad 180 \ \text{lb} \ \textbf{x} \ S_A \ = \ 90 \ \text{lb} \ \textbf{x} \ 4 \ \text{ft}$$

$$S_A \ = \ \frac{360 \ \text{lb-ft}}{180 \ \text{lb}}$$

$$S_A \ = \ 2 \ \text{ft}$$

So individual A must sit closer to the fulcrum (i.e., A's moment arm is shorter than B's moment arm).

Note: The relationship between force, moment arm, and torque is fundamental to your understanding of bones and muscles as lever systems. Before proceeding, you should review the seesaw example just presented and be sure that you understand the mechanical concepts involved.

UNITS OF FORCE AND TORQUE

In the preceding example, force is given in pounds and torque is expressed in pound-feet. Note that the lb-ft is not the same as the ft-lb (foot-pound), a unit of work which is different from torque.

In this laboratory, force and torque will be expressed in metric units in an absolute system using the kilogram, meter and second as the fundamental units of mass, length and time, respectively (see Appendix 1). A force is a push or a pull on an object which imparts an acceleration to the object. The magnitude of a force depends on the mass of the object and its acceleration. That is:

Force = mass **x** acceleration

The **newton** (abbreviated, N) is the force that will give an acceleration of 1 m/sec^2 to a mass of 1 kg. The weight of an object, which is also a force, is the product of the object's mass and acceleration caused by the earth's pull (gravity) on the mass. The earth's gravity produces an acceleration of 9.8 m/sec^2 on an object. Therefore, the weight of a 1 kg mass is:

1 kg **x** 9.8 m/sec^2 = 9.8 **newtons**

Since weight is a force, it is expressed in the unit of force. The following approximate equations may help you relate the metric units of mass and force to the pound, a more familiar unit of force to most of us living in the United States.

1 kilogram ~ 10 newtons ~ 2.2 pounds
1 newton ~ 0.22 pound
1 pound ~ 4.5 newtons

Torque is expressed in units force - length. The appropriate units for this laboratory are **newton-meter** (abbreviated, N-m).

BONE/MUSCLE SYSTEMS AS LEVER MACHINES

Let us now formalize the concepts of in-torque and out-torque derived from our seesaw example by considering the use of levers in vertebrate skeletal systems. A typical skeletal lever system involves two bones (Figure 9.3). Bone 1 serves as the lever upon which forces are exerted, while bone 2 provides the fulcrum about which bone 1 turns. Notice that the lever system in Figure 9.3 is the same as the seesaw in Figure 9.2, except it is inverted. A muscle inserted on bone 1 contracts and produces the in-force (F_i) on the lever system. (Note that because a muscle can produce a force only when it contracts, F_i is directed upward in this case, unlike the downward-directed force applied by the weight of a person in our seesaw example). The input-moment arm (S_i) is the distance separating F_i from the fulcrum. The force of the muscle's contraction in combination with the input-moment arm acts to produce a clockwise in-torque (T_i) that is equal to F_iS_i.
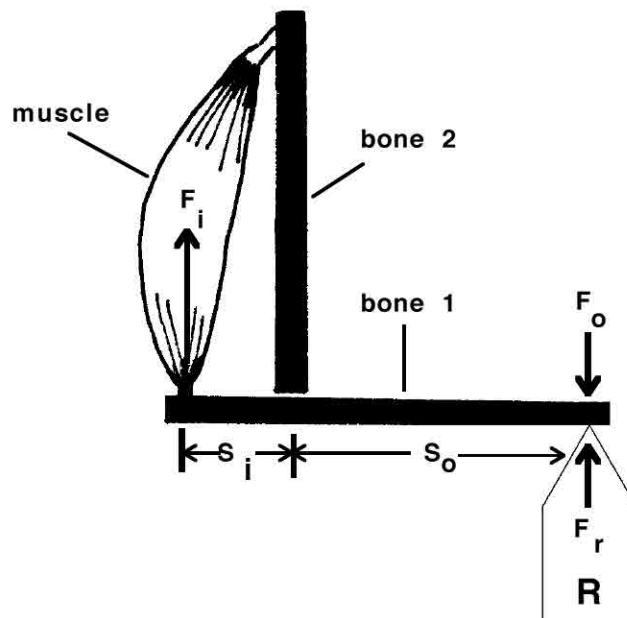
Figure 9.3. An endoskeletal lever system. See text for discussion.

When an in-force (F_i) is applied to bone 1 at a position left of the fulcrum, the bone has an out-force (F_o) anywhere to the right of the fulcrum. The out-force is the force created by the tendency for bone 1 to pivot about the fulcrum and move (in this case clockwise) when F_i is applied. The output-moment arm (S_o) is the distance between the fulcrum and the position on bone 1 at which it applies the out-force (F_o)

The out-torque (T_o) is, therefore, equal to F_oS_o. Note that T_i and T_o are equal in both magnitude and direction of rotation. Convince yourself that the following relationships hold true for the situation diagrammed in Figure 9.3:

$$\text{in-torque} = \text{out-torque}$$

$$T_i = T_o$$

$$F_iS_i = F_oS_o$$

F_o can now be used to push against some environmental resistance (R), which is equivalent to the second person in our seesaw example. According to Newton's laws of motion, as force is applied by the lever system against R, a force equal in magnitude but opposite in direction will be applied by R against the lever system. This resistance force (F_r) increases directly with F_o and sets up a counterclockwise torque (a resistance torque) equal to F_rS_o (which also equals F_oS_o). As long as R does not move and the lever does not rotate on its fulcrum, the lever system is in equilibrium. As predicted by the principle of leverage, at equilibrium the clockwise torque = counterclockwise torque, or

$$\text{in-torque} = \text{resistance torque}$$

$$T_i = T_r$$

$$F_iS_i = F_rS_o$$

If the muscle in our lever system is capable of contracting with sufficient force to generate an F_o that equals or exceeds the force required to move R (i.e., $F_o > F_r$), bone 1 will rotate on bone 2 in a clockwise direction and push R with it. In this situation the lever system has done useful work. When R moves, the system is no longer in equilibrium because the clockwise torque does not equal the counter-clockwise torque. It is important to remember that while T_i always equal T_o, whether the lever moves or not depends on whether T_o exceeds any resistance to movement. At equilibrium, T_o is balanced by the torque generated by a resistance that is counter in effect to T_o. The proportionality constants are the ratios of their moment arms.

$$\text{Since } T_i = T_o, \; F_iS_i = F_oS_o$$

$$\text{Therefore, } F_i = \frac{S_o}{S_i} F_o \text{ and } F_o = \frac{S_i}{S_o} F_i$$

It is important to remember that no matter what the condition of the lever system, T_i is <u>always</u> equal to T_o. However, only in the equilibrium state (i.e., no motion of the lever) does clockwise torque = counterclockwise torque.

The Classes Of Levers

The three recognized classes of levers are illustrated in Figure 9.4. Also shown are examples of how the human foot/ankle system and three commonly used tools can function as each class of lever. <u>You are not required to memorize the classes of levers</u>. Rather, learn to identify the fulcrum, the in- and out-forces, and the corresponding moment arms. For example, in levers of the first class, the input and output forces are applied on opposite sides of the fulcrum, whereas in levers of the second and third classes, the forces are applied on the same side of the pivot point, although in different positions relative

to the fulcrum. To hold the lever steady, the out-force must balance the resistance force, or load. For the lever to do useful work, the out-force must overcome the resistance force.

The class of lever employed often depends upon the type of movement intended. For example, all three types of levers may be found in the movements of the human foot. Depressing the accelerator pedal of a car with your toes involves the use of a first class lever; standing on your toes involves a second class lever if we focus our attention on your straining calf muscles; and lifting a soccer ball with the upper surface of your foot involves a third class lever when we consider the muscle activity of the shin area.
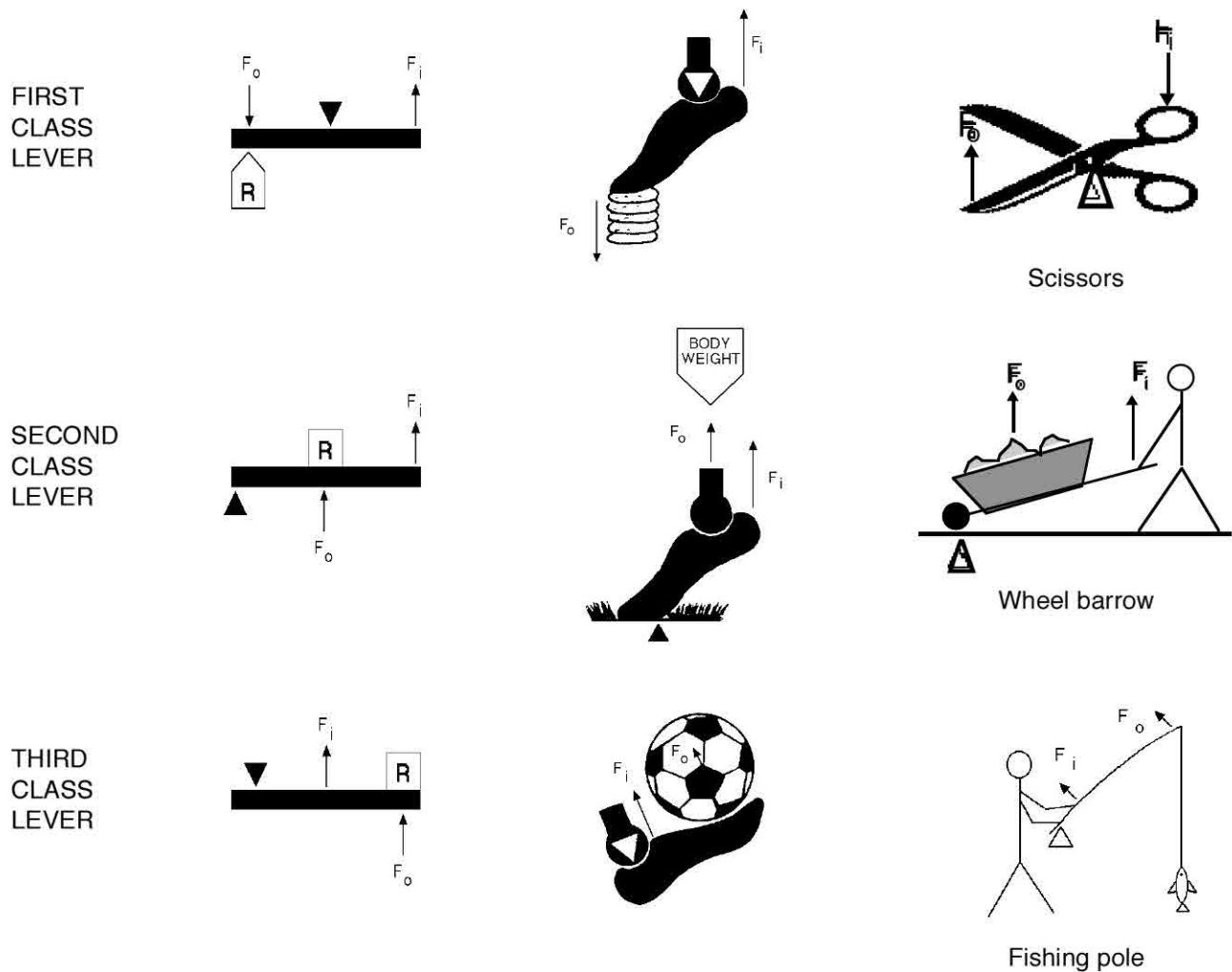
Figure 9.4. The three classes of levers as found in the movements of the human foot/ankle system and three commonly used tools. F_i = in-force; F_o = out-force. R represents the resistance against which the out-force is applied. The fulcrum is at the point of the triangle. (Adapted from Hildebrand, 1988).

Review of levers, forces, and torques

1.   Application of an in-force to a lever system always creates an out-force at some other position on the lever.

2.   Torque is generated when an in-force is applied to a lever which is capable of rotating about a fulcrum. This torque is equal to the product of the force's magnitude times the length of the moment arm (i.e., $T = F \times S$).

3.   The in-torque created by the application of an in-force <u>always</u> produces an equivalent (in both magnitude <u>and</u> rotational direction) out-torque at some other position on the lever (i.e., $T_i = T_o$).

4.   When two in-forces are applied on opposite sides of a fulcrum, torques of opposite rotational direction are generated. In the special case of equilibrium (no motion about the fulcrum), the magnitude of these torques is equal (i.e., $T_{cw} = T_{ccw}$). Motion is achieved (i.e., the lever does useful work), when the in-torque exceeds the resistance-torque (i.e., $T_i > T_r$).

DAY ONE - THE KINEMATIC MODEL

       Kinematics is the branch of physical science that studies motion. Simplified mechanical models are often used in kinematic studies to illustrate the principles of motion which we have just discussed. In order that you may explore these basic principles in detail, you will do a series of exercises that employ a physical model of the lower leg and foot of a vertebrate. Although simplified, the model exhibits all of the relationships among forces, moment arms and torques that have emerged from our discussion of leverage. Figure 9.5 shows a diagram of the kinematic model and the muscle/skeletal lever system it represents.
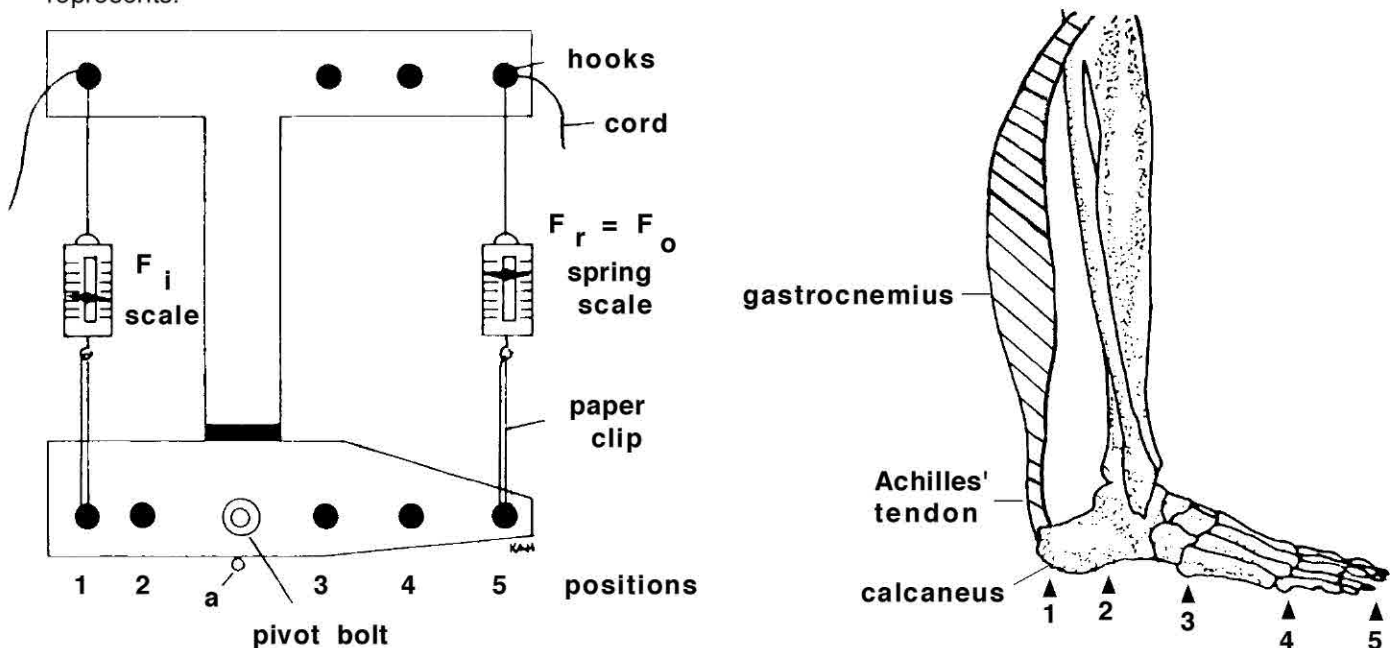
Figure 9.5. The kinematic model (left) and the human lower leg and foot (right). An eye-ring is attached at position "a" on the model. Positions 1 and 2 represent attachment points for the tendon of the gastrocnemius muscle, whose contraction produces an in-force on the calcaneus (measured by the spring scale). Positions 3, 4, and 5 represent points on the front of the foot where an out-force can be measured. The spring scale measures the resistance force needed to balance the out-force generated by the foot at positions 3, 4, and 5.

Positions 1 and 2 on the model represent attachment sites for the Achilles tendon to the heel bone (calcaneus). The Achilles tendon provides the insertion for the extensor muscles of the foot. The major foot extensor in all vertebrates is the gastrocnemius, but other muscles may also be involved in some vertebrate groups. A contraction of the extensor muscle is simulated by applying tension to the spring scale to produce a measured in-force on the calcaneus. Positions 3, 4 and 5 are points on the front of the foot where an out-force can be measured with another spring scale.

Use Of The Kinematic Model

In all the exercises using the kinematic model assume you are modeling the lower leg and foot of two different species. Species 1 has its foot extensor inserted at position 1, while in species 2 the foot extensor is inserted at position 2.

❑   Work in groups of three if possible. Use the following sequence of procedures in manipulating the kinematic model.

1.   Each group obtain a kinematic model, 2 spring scales with cords and one scale without a cord.

2.   Each member take a scale and observe the plastic rod attached to the spring. Note that the wider edge of a tapered disc on the rod indicates the force applied to the scale. The wider edge of the disc should indicate zero on the **N** scale when the scale is held vertically and no force is applied; if it does not, adjust it until it does by turning the nut on one end of the scale.

3.   Check the tightness of the nut on the bolt making the ankle joint of the model. The nut should be tight enough to eliminate excessive wobble between the foot and the leg, but loose enough to allow the foot to rotate freely on the bolt.

4.   Lay the model flat on the bench top. Attach 2 spring scales to the eye screws on the foot at position 1 and 5 as shown in Figure 9.5. Be sure the newton (N) scale is showing. Pass the cords over the hooks on the top bar (do not tie the cords or loop them more than once around the hooks).

IMPORTANT NOTICE for all subsequent steps: When forces are applied and measured on the model, the foot must be nearly perpendicular to the leg. If the foot is too far from perpendicularity with the leg, it may be jammed against the leg and unable to rotate freely.

5.   One person holds the model down by the leg being careful not to interfere with the spring scales. The other two persons manipulate and read the scales. In all cases the person with the F_i scale (at positions 1 or 2) should apply an input force of 20 N while the person with the F_r scale (at position 3 or 5) applies a resistance force to keep the foot perpendicular to the leg.

To do this, concurrently apply forces to both ends of the foot by pulling on the cords attached to the scales until an in-force of 20 N is achieved. Now, with no further adjustment in the F_r scale, increase the F_i slightly beyond 20 N and then gently decrease the F_i to exactly 20 N and read F_r on its scale.

Basic Principles Of Leverage

1.   **Torque**: Use the kinematic model to verify that at equilibrium, clockwise torque = counterclockwise torque, and that for any lever system, $T_i = T_o$.

❑   With the spring scales attached to positions 1 and 5 and the foot perpendicular to the leg, apply a 20 N in-force at position 1 and measure the out-force generated at position 5. Verify that the foot is stationary relative to the leg (i.e., is at equilibrium) only when the two forces exert equal but opposite torques about the fulcrum (the ankle joint). Rulers are available to measure lever arms (measure to the nearest mm and convert to meter).

$F_i = 20$ N $S_i = $ _____ $F_o = $ _____ $S_o = $ _____

$T_i = F_i S_i = $ _____ $T_o = F_o S_o = $ _____

❑   Move the out-force scale to position 3 and its cord to the hook above that position. Apply a 20 N in-force at position 1. Is the magnitude of the force needed to produce a balancing torque changed? Calculate T_i and T_o. Are the torques still equal?

$F_o = $ _____ $S_o = $ _____

$T_i = F_i S_i = $ _____ $T_o = F_o S_o = $ _____

2.   **Mechanical Advantage (MA)** is the ratio of the out-force of a lever system to the in-force needed to generate it.

$$MA = \frac{\text{out-force}}{\text{in-force}} = \frac{F_o}{F_i}$$

Different species of animals have different bone lengths and insertion points for muscle attachment. Use the kinematic model to consider how the four factors of leverage (F_i, F_o, S_i, S_o) affect the mechanical advantage of various skeletal arrangements.

The procedure described above modeled an animal that has its muscle inserted at position 1.

❑   Enter in Table 9.1 the out-forces generated at positions 3 and 5 in this model.

❑   Now model an animal with its foot extensor inserted at position 2. Again, using a 20 N in-force, measure the out-forces generated at positions 3 and 5. Enter these data in Table 9.1.

Table 9.1. The out-forces (F_o) generated at positions 3 and 5 given a constant in-force (F_i) of 20 newtons at position 1 or 2.

		Out-force at position:	
		3	5
In-force of 20 newtons at position:	1		
	2		

? At which <u>output position</u> does the 20 N in-force generate the greater out-force? _____ At which <u>muscle insertion point</u> does the 20 N in-force generate the greater out-force? _____

? Look again at the equation $F_i S_i = F_o S_o$. If F_i and S_i are constant, how does F_o relate to S_o?

We can gain insight into the force conversion efficiency of the leg/foot lever system by estimating the **mechanical advantage** (MA) of the machine. The out-force generated at the foot positions 3 or 5 is divided by the in-force (the constant 20 N force generated by the muscle).

❑ Using your data from Table 9.1, calculate the MA produced at the two output positions for an animal species with its muscle inserted at position 1. Enter these data in Table 9.2. Do the same for a second species with its muscle inserted at position 2.

Table 9.2. Calculated MA for various positions of F_o and F_i.

| | Out-force position: | |
	3	5
In-force position: 1		
2		

? For both animal species (foot extensor inserted at position 1 or 2), at what point along the foot is the greater MA observed (e.g., position 3, the proximal metatarsals, or position 5, the toes)?

_____

? Which of the two species has the greater MA? _____

? During the evolution of a population of animals, what skeletal parts of a foot may change to increase the mechanical advantage of the foot extensor muscle system?

3. **Speed Ratio (SR):** The kinematic model can also be used to show how the various muscle attachment positions and bone lengths of different animal species create skeletal arrangements with different speed ratios. Speed is the rate of movement (the distance moved per unit of time). When a muscle contracts, it is shortened by a certain distance in a given period of time. Since the distance of muscle contraction is equal to the distance moved by the bone at the muscle's insertion point (= in-force application point), we shall call the rate of movement of the in-force point on the bone the <u>input speed</u>. In a bone-muscle lever system, contraction of the muscle causes the out-force position on the bone to move. We shall call the rate of movement of the out-force position the <u>output speed</u>.

The output to input **speed ratio (SR)** of a bone-muscle lever system is the ratio of the speed of the out-force position to the speed of the in-force position on the bone during contraction of the muscle.

SR = <u>speed of out-force position</u> = <u>distance moved by out-force position/time</u>
**   speed of in-force position   distance moved by in-force position/time**

Since both the out-force and the in-force positions are moving in the same period of time, SR may be expressed as follows:

SR = distance moved by out-force position
distance moved by in force position

Let O = distance moved by the out-force position
and M = distance of muscle contraction = distance moved by in-force position

Then: **SR = O**
M

❑   To obtain estimates of SR, you need to measure the arcs created by points corresponding to the two muscle insertion positions and two of the three foot out-force positions (positions 3 and 5) as they rotate around the ankle joint. Make the arcs by placing the model on a sheet of paper with pencils positioned in holes 1, 2, 3, and 5 along the foot. Make sure to move the foot through the full extent of its rotation. <u>Carefully mark the position of the pivot bolt</u>. Measure (to the nearest mm) the moment arms and chords of the arcs you create at all four positions (see Figure 9.6). M_1 and M_2 are the contraction distances for extensor muscles inserted at distances S_1 and S_2 from the fulcrum. O_3 and O_5 are the distances moved by out-force positions 3 and 5, respectively. Record your data in the column and row headings of Tables 9.3 and 9.4 respectively.
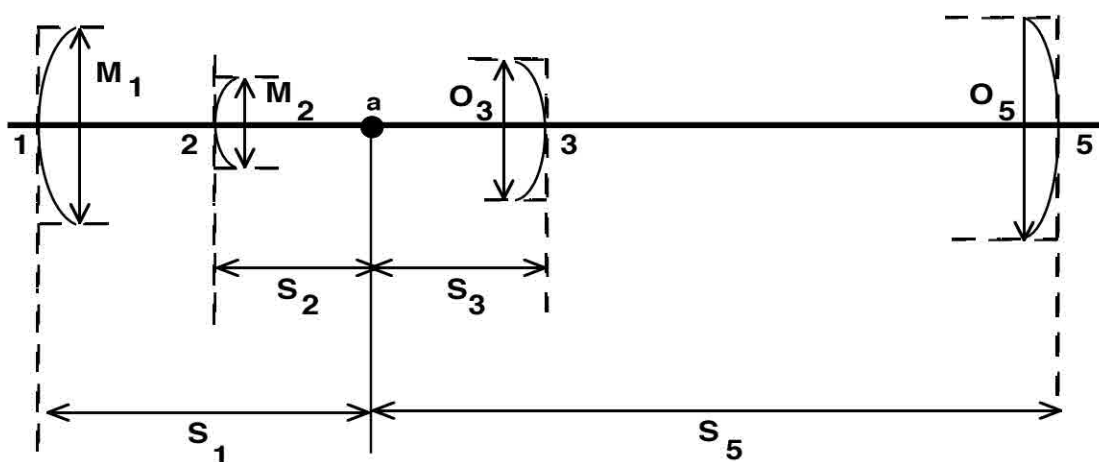
Figure 9.6. The relationship between moment arms (S_1- S_5) and the corresponding distances of muscle contraction (M_1 and M_2) and foot out-force position movement (O_3 and O_5). a is the axis of rotation.

❑   Calculate speed ratios (SR = O/M) from your measurements of O and M and enter these values in Table 9.3.

Table 9.3. SR computed from the ratio O/M, the distance moved by the out-force position of the foot relative to the distance the muscles contracted.

Movement of Out-force Position

	chord lengths	$O_3 =$	$O_5 =$
Muscle Contraction Distance	$M_1 =$		
	$M_2 =$		

In a living animal, it would be nearly impossible to measure changes in muscle length (M) as the animal moves. If all that you have available is a skeleton of the animal, muscle movement is impossible to measure directly. Therefore, you need to find a relationship that is proportional to O/M and easier to measure.

❑   Enter the ratios of S_o/S_i in Table 9.4 and compare these ratios to the SR values recorded in Table 9.3.

Table 9.4. S_o/S_i ratios calculated from two different output moment arm lengths and two different input moment arm lengths.

Out-force Position

	moment arm lengths	$S_3 =$	$S_5 =$
In-force Position	$S_1 =$		
	$S_2 =$		

?   Why should a relationship exist between SR and the corresponding moment arm ratios?

?   What general formula can you give for SR in terms of moment arms? SR = _____

?   Look at the SR values for the two muscle insertion positions and the two out-force positions. How does the SR of a species with its muscle inserted at position 1 compare with that of a species whose muscle insertion is at position 2?

?   Consider two species whose extensor muscles insert at an equal distance from the axis of rotation, but whose feet are of different lengths. Which species would have the greater SR?

? What part of your foot contacts the ground when you want to run with high speed (as in sprinting)? Does your leg/foot lever system agree with what would be predicted from your measurements with the kinematic model? How?

? Manipulate the law of the lever equation to express mechanical advantage and speed ratio as ratios of forces and ratios of moment arm lengths.

$$F_i S_i = F_o S_o \qquad\qquad MA = \qquad\qquad SR =$$

? What is the relationship between MA and SR for any muscle/skeletal lever system?

4. **Compression Forces:** This exercise will demonstrate the compression forces that develop at the pivots (articulation surfaces) of skeletal lever systems.

❑ Remove the pivot bolt from the ankle joint and attach a third scale to the eye-hook (labeled **a** in Figure 9.5). While one or two persons firmly hold stationary the upper two spring scales (muscle scale attached to position 1, force output scale attached to position 5), another person pulls down on the joint scale. Simultaneously read all three scales. The joint scale measures the compression force (F_c) that F_i and F_r develop at the pivot point. (Recall that when the lever is in equilibrium, $F_o = F_r$).

$$F_i = \underline{\hphantom{XXXX}} \qquad\qquad F_o = \underline{\hphantom{XXXX}} \qquad\qquad F_c = \underline{\hphantom{XXXX}}$$

? What is the relationship between F_c, F_i, and F_o? Is this relationship still observed if F_i and F_r are applied at other positions on the model? Try it!

❑ When you are finished with compression force measurements, reassemble the model with the washers in their appropriate positions.

❑ Based on the relationship you observed above, calculate an estimate of the compression force (F_c) exerted on the pivot point for each of the in-force and out-force combinations in Table 9.1. Enter your estimates in Table 9.5

Table 9.5. Estimated compression force (F_c) generated at the pivot (ankle) joint for different positions of F_i and F_o. F_i = 20 N.

Out-force position:

In-force position:		3	5
	1		
	2		

?   Which combination of muscle insertion and bone output length produces the greatest compression force? _____

Which lever system would be subjected to greater stress at the joint?

a.   a lever system adapted for speed

b.   a lever system adapted for force output

?   What structural adaptations would be useful in dealing with the compression forces generated at the articulations of skeletal lever systems?

?   What material covers the actual articulation surfaces between bones in a vertebrate skeleton? What is the function of this material?

DAY TWO - ANALYSIS OF ARTICULATED VERTEBRATE SKELETONS

Read Hildebrand's (1974) article "How Animals Run" <u>before</u> coming to lab. In this article, the horse and cheetah are used extensively as examples of vertebrates capable of rapid locomotion. Compare and contrast the skeletons of these two animals with respect to the characteristics that make them good runners.

Now that you have developed some insight into the movement of levers and how they transmit force, and the adaptations of bone-muscle lever systems to achieve high force output or high speed output, you can apply this knowledge to the analysis of the articulated skeletons of a few vertebrates. Attempt to correlate structure and function as you study the skeletons. Skeletons of four familiar vertebrates—frog, bird, cat and human—and one less familiar mammal—the mole—are available for study. Figures of these skeletons, with many of the bones identified, are included in this chapter to assist you. Use the questions and comments that accompany each figure as a guide to your study. Adaptations for locomotion and other uses of the appendages are emphasized in this study. Look for prominent bumps, knobs, ridges and extensions on bone. These protuberances are attachment sites (origins and insertions) for skeletal muscles.

Adaptations for Running and Other Rapid Limb Movements

The speed at which an animal walks or runs depends on the <u>length</u> and <u>rate</u> (i.e., number per time unit) of its strides. A stride is one complete cycle of motion in the locomotory process. The running legs of cursorial animals generally are relatively long, compared to the animal's size, to provide a long stride. Another adaptation for running, and also for jumping and flying, is a reduction in weight of the locomotory limbs so they can be moved more easily and, consequently, more rapidly.

Definition of Terms

In your studies, references will be made to the <u>pectoral</u> and <u>pelvic</u> girdles, and a <u>trunk</u> or <u>torso</u>, which are defined below.

Pectoral girdle: arch which supports the front limbs. It consists of:
sternum (breastbone)
clavicles (collarbones) or furcula (wishbone) in birds
scapulae (shoulder blades)
coracoids in the frog and the bird

Pelvic girdle: arch which supports the hind limbs.

Trunk = **Torso**: animal body apart from the head and appendages.

NOTE: You are not required to learn the names of bones and muscles. Some names will become familiar to you through their repeated use.

❑ The entire laboratory class works as one group to study the human skeleton, and then divides into four groups. Each group studies a particular skeleton and then progresses to another until the group has studied all four of the other skeletons.

PHALANGES

METACARPALS

CARPALS (WRIST)

SKULL

CRANIUM

MAXILLA

MANDIBLE

RADIUS

ULNA

AXIAL SKELETON

APPENDICULAR
SKELETON

CERVICAL VERTEBRA

CLAVICLE (COLLAR BONE)

STERNUM (BREASTBONE)

RIB

THORACIC VERTEBRA

SCAPULA
(SHOULDER BLADE)

HUMERUS

LUMBAR VERTEBRA

SACRUM (RUMP)

ILIUM

COCCYX
(TAILBONE)

PUBIC ARCH

ISCHIUM

FEMUR (THIGH)

PATELLA (KNEECAP)

TIBIA (SHINBONE)

FIBULA (CALF BONE)

TIBIA

FIBULA

CALCANEUS (HEEL)

TARSALS (ANKLE)

METATARSALS

PHALANGES

Figure 9.7. Skeleton of a human (*Homo sapiens*).

HUMAN

Note: As you examine the human skeleton, you may carefully manipulate parts of it to see the movement of bones at joints. If a skeletal part does not move easily, do not apply excessive force to move it. If you do, you may break the skeleton. Notice that cartilage pads are absent from bone surfaces in joints. In many joints the spacing and alignment of bones is not as it would be in the skeleton of a complete human.

❏ Experience the range of motion you have in your arms by extending them above your head, to the sides, behind and in front of your body. Cross your arms while they are extended behind and in front of your body.

The arrangement of bones composing the pectoral girdle and the type and location of shoulder joints allows this range of motion.

" The ball-and-socket joint between the **humerus** and **scapula** (shoulder blade) allows a large range of motion.

" **Scapulae** lie more-or-less flat across the dorsal side of the ribcage which puts the shoulder joints outermost on the pectoral girdle.

" The ribcage is wide, but not deep, so there is minimal obstruction for crossing and extending the arms on the ventral side of the body.

The skeletal characteristics listed above are adaptations for locomotion by brachiating--swinging by the arms, hand over hand. Other adaptations for brachiating are:

" A rigid frame in the pectoral girdle formed by the **clavicles**, each attached at one end to the **sternum** of the ribcage and at the other end to a **scapula** at the shoulder joint. This frame, bound together with ligaments and muscles, provides a structure strong enough to hold the body together when most of its weight is hanging by one arm.

" The large surface area of the **scapulae** provides many attachment sites for tendons of the back muscles needed for brachiating (i.e. some of the muscles you use in doing pull-ups).

The rigid frame of the pectoral girdle also enables us to keep our shoulders in place when we lift heavy objects with our arms.

? What adaptations for brachiating are present in the human hand?

Humans are the only species of vertebrates that generally walk and stand with the torso erect above the hind legs. The structure of the pelvic girdle and its musculature enables us to do this. The human pelvis is relatively shorter and wider than that of other primates. The **ilium** of the pelvis is large and projects laterally over the hip joint. The ilia and other parts of the pelvis provide attachment sites for many of the muscles involved in walking, running and maintaining an upright torso.

❏ Stand flat on both feet and bend your torso backward. While doing this, feel the tightness in the muscles on the back of your thigh and your buttocks muscles.

The muscles you felt on the back of your thigh are collectively called hamstring muscles. Some of them are attached to the **ischium** of the pelvis and to the bones of the lower leg just beyond the knee. If the lower leg is free to move, contraction of the hamstring muscles flexes the lower leg.

❑   Stand on one foot, flex the lower part of your free leg and feel the tension in the tendon of the hamstring muscle just above the knee.

The muscles you felt on your buttocks and hip are the gluteal muscles. Gluteus maximus, the buttocks muscle, is attached to the **ilium** and **coccyx** and the dorsal side of the **femur**. The gluteal muscles of the hip are attached to the **ilium** and to the side of the **femur**.

❑   Stand on one foot and:
    1.   move your free leg dorsal (pull it backward) and feel, with your hand, the contraction of your buttocks muscle.

    2.   move your free leg out to the side and feel the contraction of your hip muscles.

❑   Stand flat on both feet and feel, with your hand, the tension in the muscles of both hips. Lift one foot and balance yourself on one leg without a balancing aid. While doing this, feel the tension in the hip muscles of your supporting leg.

?   When you did this last exercise, you were using a lever system similar to a seesaw in your body.

    1.   What was the fulcrum?

    2.   What was the lever?

    3.   What produces the input force?

    4.   What provided the resistance force?

The gluteal and hamstring muscles attached to the pelvis are involved in moving certain parts of the legs in walking and running. These muscles also are used to maintain an upright position of the torso (i.e., prevent it from jackknifing on the legs) during standing, walking and running. Furthermore, the ability of these muscles to straighten a ventrally inclined torso when the feet are fixed enables humans to perform one of their other unique procedures--to flex the body at the hips, pick up a relatively heavy weight, lift it by straightening the body and carry it ventrally in an upright position.

?   Why is a relatively flexible spine adaptive for humans?

FROG

The posture of the mounted frog skeleton is different from the way it would normally be in a sitting, live frog. The head and torso of a live frog would be higher from the ground, with the anterior end higher than the posterior end. The entire pectoral girdle has been moved toward the vertebral column giving the skeleton a squashed appearance in the chest region (see Figure 9.8).

Frogs are highly specialized for swimming and jumping and show few of the locomotory characteristics of their ancient ancestors. For example, they do not exhibit the sinusoidal motion of salamanders (and presumably of ancient amphibians) during swimming and walking. The limited number of articulating vertebrae in the frog restricts this type of motion.

❑ Study the pelvic girdle and its limbs.

The most notable characteristic of the skeleton is the relatively long hind limbs. Important features of these limbs are:

" The lower leg bone, the **tibiofibula**, is a single, strong bone resulting from the fusion of the tibia and the fibula.

" The foot is lengthened by the elongation of the toe bones and the ankle bones.

" The ankle, called the **tarsus**, consists of only two bones, one of which is the calcaneum (similar to the calcaneus in the human).

" Neither ankle bone projects behind the foot at the ankle joint. Consequently, the foot has a large angle of rotation at this joint. The tendon of the gastrocnemius, the major foot extensor muscle in the lower leg, passes over the ankle joint and inserts just beyond it on the bottom of the foot. Contraction of the gastrocnemius causes the push of the foot for swimming or jumping.

? Is this foot lever system adapted for high speed or high force output?

? Consider the mechanical advantage of this foot lever system. How does the frog produce enough out-force in the extension of its foot to push it into a long jump?

? Is the foot the only lever system involved in jumping?

The anterior ends of the pelvic girdle bones are attached to the ends of lateral projections on the last (most posterior) vertebra to form a rigid frame. The sockets of the hip joints are in the posterior end of the frame. A middle brace, the **urostyle**, runs lengthwise through the frame to strengthen it. (The urostyle may be disjointed at its posterior end on your skeleton.) The urostyle is regarded as a structure resulting from the fusion of several vertebrae.

? What is the probable function of the large bone surfaces on the sides and posterior end of the pelvis?

When a frog jumps, it is rapidly pushing a projectile into the air. A rigid projectile is easier to launch than a flexible one is because the rigid body does not dissipate energy by wiggling. The posterior half of a frog's body trunk is supported by the rigid pelvis. The anterior half of the trunk is supported by a relatively inflexible vertebral column and the rigid frame of the pectoral girdle. Thus, when a frog jumps, the pushing action produced by the rapid extension of legs and feet is transmitted directly to the rigid frame composed of the pelvic girdle and urostyle and then to the relatively rigid vertebral column, which carries the pectoral girdle.

The joints between the pelvic girdle and urostyle and the last vertebra form a hinge between the spinal column and the pelvis which allows much dorso-ventral rotation. This bending in the middle of the back is thought to act as a shock absorber during a landing from a jump.

❑ Observe both the dorsal and ventral sides of the skeleton to see all of the pectoral girdle.

The pectoral girdle of the frog consists of more bones than that of any other skeleton you will observe in this study. The major features of the pectoral girdle are:

" The **sternum** (breastbone) consists of four bones arranged in a row.

"   The **scapulae** (shoulder blades) lie on the sides of the girdle. A pair of **clavicles** (collar bones) and a pair of **coracoids** (not present in mammals) are connecting braces between the scapulae and the sternum.

"   A thin broad bone, the **suprascapula**, is attached dorsally to each scapula. Suprascapulae are not present in birds or mammals.

Since the frog does not have ribs, it has no ribcage to protect certain vital organs. Protection of some organs in the thoracic region is provided dorsally by the lateral projections of the vertebrae, laterally by the suprascapulae and scapulae and ventrally by the sternum and coracoids.

The major locomotory role of the front limbs is participation in quadrupedal walking. They also raise and support the anterior part of the frog, so its torso is inclined for jumping. They function mainly in steering when the frog is swimming, and they provide support and absorb the impact when the frog lands on a firm surface after a jump. The rigidly braced construction of the pectoral girdle may be necessary to withstand the impact of landing from a jump.

BIRD

A skeleton of a chicken or a pigeon is available for viewing. Please handle the skeleton very carefully; it is quite delicate. Some of the smaller bones may be missing.

❑   Study the arrangement of the bones composing the pectoral girdle.

The most conspicuous bone is the **sternum** (breastbone) with its prominent keel. The **scapulae** (shoulder blades) are long, slender bones which lie parallel to the vertebral column on the dorsal side of the ribcage (they may be partially hidden by the large wing bones). The scapulae are connected to the sternum by the **coracoids** which make the pectoral girdle strong and relatively rigid. Coracoids are not present in mammalian skeletons. Some of the ribs form braces between the vertebral column and the sternum to strengthen the pectoral girdle (see Figure 9.9).

The slender V-shaped bone which attaches to the coracoids near the shoulder joints is the **furcula**, commonly called the wishbone. The furcula is assumed to be a bone resulting from the fusion of two clavicles (collar bones). The function of the furcula is not clearly understood. A current hypothesis is that it functions as a vibrating spring to regulate breathing when the wings are beating. A few flight muscles have their origins on the furcula.

What is the function of the keel on the sternum and why must the pectoral girdle be strong and rigid? The keel and other parts of the sternum provide the attachment surfaces (origins) for most of the flight muscles, the biggest muscle mass in flying birds. These muscles are attached by tendons to the **humerus** of the wing near the shoulder joint.

❑   Note the following features of each humerus:

1.   a large knob (or tuberosity) near the shoulder joint,

2.   a ridge near the knob but slightly farther away from the shoulder joint.

The pectoralis major, the main muscle to depress the wing and provide the power stroke for flight, inserts on the large knob of the humerus. Contraction of this muscle pulls the wing down.

Unlike humans or bats (flying mammals) which use back muscles to pull back arms or elevate wings, birds use a breast muscle to elevate the wing. The tendon of this muscle passes through an opening called the **triossial** (3 bones) **canal** at the junction of the **scapula, coracoid** and **furcula** and inserts on the ridge of the humerus. This arrangement of parts functions like a pulley (a type of lever) which enables the ventrally directed force resulting from muscle contraction to produce a dorsally directed force to lift the wing.

Unfortunately the bones which join to form the triossial canal and shoulder joint are misaligned in some of the bird skeletons, so their pulley arrangement cannot be seen.

? Consider the fully extended wing with feathers to be a lever with the flight muscles providing the input force.

Are the in-force and out-force on the same side or opposite sides of the fulcrum?_____

Is this muscle-lever system adapted for speed output or force output?_____

If you correctly answered the question above, you realize that a very large in-force from muscle contraction is required to produce the force to depress the wings in the power stroke for flight. The contraction of the flight muscles pulls the shoulders toward the breast. The **coracoid** braces are essential for flying birds because they immobilize the shoulder sockets so that the full contractile force of the flight muscles is applied to the wings, and there is no loss of muscular force by the ventral displacement of the shoulders.

❑ Examine a wing of the skeleton. Locate the wrist and the elbow.

The major adaptation for flight in the forelimb is a reduction in the number of bones. From the shoulder to the wrist, the bird's wing is like that of many other vertebrates; it consists of a **humerus** joined at the elbow to an **ulna** and a **radius**. The human wrist has eight carpals; the bird's wrist has only two carpals. Two other bones in the bird's wrist, together called the **carpometacarpus**, are the result of a fusion between carpals and metacarpals (palm bones). The bird wing has three digits (fingers) each composed of only one or two bones.

❑ Observe a hindlimb of the skeleton. Identify the knee and the ankle.

The bird's leg shows adaptations for high speed output. One adaptation--a reduction in the number of bones--reduces weight which also is advantageous for flying. The lower leg of a bird has two bones, the tibia and fibula (as in the human), but the fibula is reduced to a slender bone which tapers to a point above the ankle joint. The bigger lower leg bone is called a **tibiotarsus** because it consists of the tibia and some ankle bones (tarsals) fused to it in the region of the ankle joint. The lower bone involved in the ankle joint, the **tarsometatarsus** is the result of fusions of ankle and foot bones; it corresponds to the arch region of the human foot. So, in the bird, one end of the foot is inclined upward and incorporated into the length of the leg's output moment arm. The bird walks or perches only on its toes.

❑ Examine the pelvis. Notice how thin it is.

The pelvis consists of bones of the pelvic girdle fused to a row of several fused vertebrae called the **synsacrum**. The large surface area of the pelvis provides attachment sites for many of the muscles of the tail and most of the thigh muscles. Recall that the thigh is the second-most meaty part of a bird.

The tail supports a fan of feathers which are positioned in various ways to enable the bird to perform a variety of maneuvers in flight. Several tail muscles are required to accomplish the adjustments in the feather fan.

The massive thigh muscles balance the body when the bird is standing by counteracting the weight of the breast muscles at its anterior end.

❑ Stand in the posture of the bird skeleton (but not on your toes) -- legs bent, torso leaning forward. Feel the tension in your thigh muscles. Keep your legs bent and lean forward as far as you can, as if you were getting something on the floor with your mouth. Raise your torso and feel where the tension occurs in your thighs. Now imagine doing these exercises with considerably more weight on your chest.

The bird's leg with its foot extension is a relatively high speed output lever. Thigh muscles inserting on the tibio-tarsus provide the in-force. Because of the low mechanical advantage of this bone-muscle lever system, large muscles whose contractions produce large in-forces are needed to provide a sufficient out-force from the toes of the foot.

❑   Notice the fused vertebrae along the dorsal side of the ribcage.

This row of fused vertebrae and the synsacrum, to which it is fused, form a rigid rod which supports the torso and the legs when the bird is in flight. Bird flight is generally smooth and graceful, not fluttery or jerky. The stability of the flying bird is due to the distribution of weight relative to the position of the wings. The shoulder joints are on the dorsal side of the torso, which puts the bird's center of weight below the wings. Thus, during the powerstroke, the wings are lifting a weight from below, which is more stable than a weight in line with or above the attachment sites of the moving wings.

CAT

Cats are carnivorous, cursorial mammals; consequently, we would expect to see adaptations for speed output in their leg-foot lever systems. With respect to number and arrangement of bones, the cat's front and hind limbs are much like those of humans. There is no reduction in the number of bones present in the lower leg, wrist, ankle or foot compared to humans, except the cat's hind foot has only four digits (toes).

❑   Using your own anatomy as a reference, identify:

the elbow: and wrist of a front limb

the knee and ankle of a hind limb

Now you should see the major differences between the limbs of cats and humans.

The **metacarpals** of the cat's front foot (the bones which form the palm region of the human hand) are relatively longer and oriented vertically to make an extension on the front lower leg, so the cat stands and runs on its front toes (see Figure 9.10).

The **metatarsals** of the cat's hind foot (the bones which form the instep region of the human foot) are also relatively longer and inclined so that only the toes are on the ground and the **calcaneus** (heel) is always raised.

The result of lengthening and inclining a portion of the foot is an increase in the effective length of the leg (the output moment arm) when the animal is running.

The muscles that move the entire leg-foot lever systems insert on the upper leg bones (i.e., the **humerus** and **femur**). Since these leg-foot/muscle lever systems are speed efficient, they have small mechanical advantages. Consequently, the muscles that move the upper leg bones must be quite large so they can produce enough in-force to give a sufficient push (out-force) from the foot to enable the animal to run. The large surface areas of the **scapulae** (shoulder blades) and the **pelvic girdle** provide attachment sites for many of the large upper leg muscles.

The leg-foot lever system of a vertebrate is not a single rigid structure, but is a series of rigid bars (each being a lever) which can move with respect to each other by rotating at the joints. The out-force (push) from the cat's foot comes not just from an in-force applied to the upper leg bone, but also from in-forces and out-forces produced in the component levers of the system.

?   Refer to your own anatomy, if necessary, to answer these questions.

Contraction of which muscles inserted on the lower front leg (ulna and radius) of the cat would produce a push from the front foot: extensors or flexors?

Contraction of which muscles inserted on the lower hind leg (tibia and fibula) would produce a push from the hind foot: extensors or flexors?

?   Can you find a structure which can be identified as the pectoral girdle?

The pectoral girdle is completely disjointed in all of the cursorial carnivores. Cats, like all mammals, lack coracoids which connect the **sternum** (breast bone) to the **scapulae**. The **clavicles** (collar bones) are very small, slender bones which are not joined to either the scapulae or the sternum. If the clavicles are present on your skeleton, they are wired to it in front of the shoulder joints. With no connectors between the sternum and the scapula, the latter has more freedom of movement anteriorly and posteriorly. The scapulae lie on the sides of a narrow but deep ribcage and are held in place by muscles originating on ribs and on the extensions of vertebrae in the neck and thoracic regions. Mounted in this way, each shoulder blade pivots at a point near the middle of its length, and the shoulder joint, at the ventral end of the scapula, is free to move forward and backward with the swing of the leg (see Figure 9.11). This arrangement increases the effective length of the tibia leg-foot output moment arm and thus increases the stride length.

?   Consider the structure of the cat's pectoral girdle and answer these questions.

Could a cat extend its front legs to its sides so they are perpendicular to its trunk? Explain.

If a cat could grasp with its front paws, could it brachiate (swing by its front legs, paw over paw)? Explain.

❑   Observe the pelvic girdle of the cat. Note that its site of articulation with the vertebral column is above the sockets of the hip joints.

The pelvic girdle can rotate somewhat at its joint with the spine. When the hind legs are flexed the pelvic girdle rotates forward, and it rotates backward as the hind legs are extended (see Figure 9.11). When the cat is running and its hind limbs are fully extended, a portion of the pelvic girdle is added to the length of the leg-foot output moment arm, thus increasing the stride length.

The very flexible spine of the cat further increases its length of stride. The trunk of a cat is considerably longer when it's back is extended than when it is flexed (see Figure 9.11). By flexing its back as the legs are drawn under the trunk, and then extending its back as it pushes against the ground by extending its hind limbs, the cat adds the increase in body length to its stride. Hildebrand (1974) proposed that a cheetah could run about six miles per hour without it's legs by using only the spring-like motion of it's back.

MOLE

Moles eat soil invertebrates such as earthworms and insect larvae. To obtain these prey moles dig burrows, sometimes just under the surface of the ground, but in some cases more than a meter underground. The entire skeleton of a mole shows adaptations for burrowing, but the pectoral girdle and front limbs display the major adaptations for generating large output forces from the muscle-lever systems.

❑   Observe the pelvic girdle and the hind limbs. Note the fusion of several sacral vertebrae with the pelvic girdle and the fusion of the two lower leg bones (tibia and fibula).

While a mole digs with its front feet, it holds itself in place by pushing its hind legs, with knees bent, out to the sides and braces its feet against opposite sides of the tunnel. The fusion of the **sacral vertebrae** with the **pelvic girdle** bones produces a structure strong enough to withstand the compressive forces generated by the two hind limbs pushing against opposing walls of the tunnel. The major function of the hind legs does not require twisting of the lower leg; consequently, two separate bones are not necessary. Note, however, that the two bones are not fused along their entire lengths; they are separated at the knee joint. The structure of the lower leg bone allows the compression force in the knee to be distributed over two bone end surfaces. The single strong leg bone in the ankle joint has a large end over which the compression force is distributed.

❑   Study the arrangement of bones in the pectoral girdle: **scapulae** (shoulder blades), **clavicles** (collar bones) and **sternum** (breastbone). <u>Note</u>: The alignment and orientation of these bones may not be exactly right on the skeleton; refer to Figure 9.12 to see a more correct arrangement of bones.

The **sternum** of the mole is lengthened anteriorly beyond the ribcage so that the pectoral girdle is positioned in line with the base of the skull. With muscles and skin attached to the skeleton, the mole appears to have no neck. The elongated sternum has a **keel** and its anterior end is thickened. The **clavicles**, are short and heavy, making the shoulder joints close together. The **scapulae** are long and thin and lie slightly inclined over the anterior ribs.

❑   Examine the front limbs. Note, in particular, the bumps and ridges on the heavy **humerus**.

The front limbs are massive relative to the rest of the skeleton. Restricted rotation of the humeri at the shoulder joints prevents them from being positioned directly downward under the animal. The mole stands and walks by doing a wide-armed push-up (see Figure 9.12A).

When the mole is burrowing, it reaches high to its front, slices down through the soil with the side of its hand and then pushes the soil to the side and behind it. During this process very little flexion or extension of the humerus occurs; the humerus remains nearly perpendicular to the long axis of the body.

❑   Attempt to mimic the digging and recovery strokes of the mole by doing the motions of a swimming breast-stroke, <u>but keep your humeri perpendicular to the sides of your body at all times</u>. Not easy, is it?

In very soft soil, a mole may dig with both limbs concurrently as you did in the breast-stroke. Usually only one arm at a time is used in the same motion.

To accomplish your awkward breast-stroke, you rotated your **humerus** about its <u>long axis</u> in one direction for the power stroke, and in the opposite direction for the recovery stroke. This is exactly what the mole does. Therefore, the input moment arm of the mole's hand-arm lever system is **one-half the width of the humerus**. The mole's humerus is very wide to make that distance relatively large. The humerus' axis of rotation is a line between the <u>elbow joint</u> of the **ulna** and the **humerus** and the shoulder joint of the scapula and the humerus (See Figure 9.12 B). The power stroke for cutting and pushing soil is accomplished by pulling the ventral (downward) half of the humerus backward causing it to rotate (see Figure 9.12 C).

The muscles involved in this rotation <u>originate</u> on the long **scapula**, the keel of the **sternum** and vertebrae in the middle of the back, and all <u>insert</u> on the lower (ventral) side of the humerus. The muscles that effect the counter rotation of the humerus (the recovery stroke) insert on its dorsal side and have origins also on the breastbone and the scapula. Notice on the skeleton how the rib cage tapers anteriorly to provide space for the large muscles that attach to the humeri and scapulae.

❑ Examine the arrangement of the ulna and radius in the wrist and the elbow. Note the long extension of the free end of the **ulna** beyond the elbow.

During the power stroke, the hand must be kept extended in line with the forelimb, and the angle between the forelimb and the humerus must be adjusted so that the push of the hand is out to the side and back as the humerus rotates. The resistance of the soil, which may be great, pushes against the hand causing it to be hyperextended and/or causing the hand-forelimb lever to flex at the elbow (see Figure 9.12 D).

Hyperextension of the hand is prevented by the arrangement of the ulna and radius in the wrist and the elbow. Rotation of the humerus in the power stroke pushes the radius forward relative to the ulna in the elbow and the wrist. Consequently, the radius pushes on the back of the hand preventing hyperextension.

Flexion of the hand-forelimb lever is prevented by producing a sufficient out-force to keep the output moment arm appropriately extended. The long free portion of the **ulna** beyond the elbow provides a relatively long input moment arm to give this lever a relatively large mechanical advantage.

❑ Study the structure of the mole's hand. Note the elongated wrist bone which projects on the thumb side of each hand.

The enlarged wrist bone increases the width of the slicing and pushing surface. Furthermore, its position on the hand places it on the cutting edge of mole excavation technology.
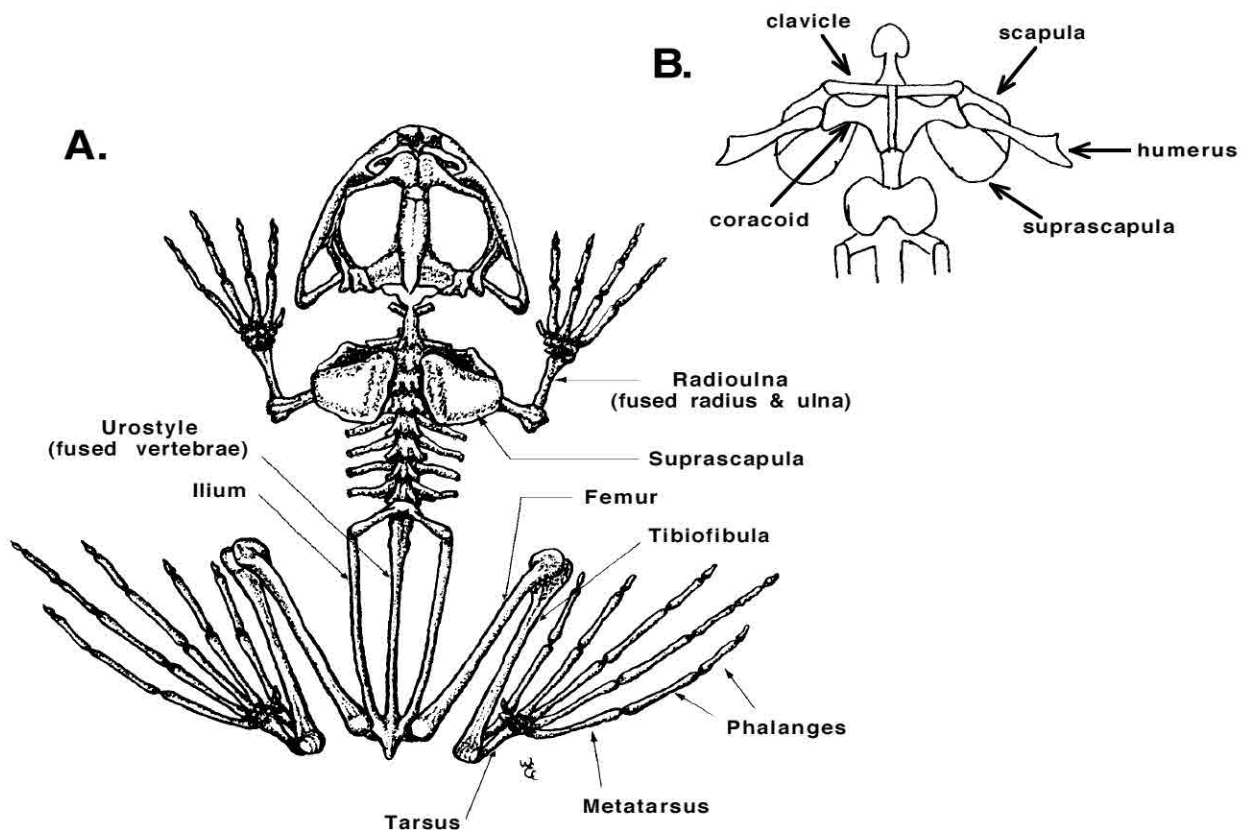
Figure 9.8. Skeleton of the grass frog (*Rana pipiens*). A. Dorsal view of the entire skeleton. B. Ventral view of the pectoral girdle. The scapulae, which are oriented vertically between the coracoids and the suprascapulae, are not easily seen in these two views.
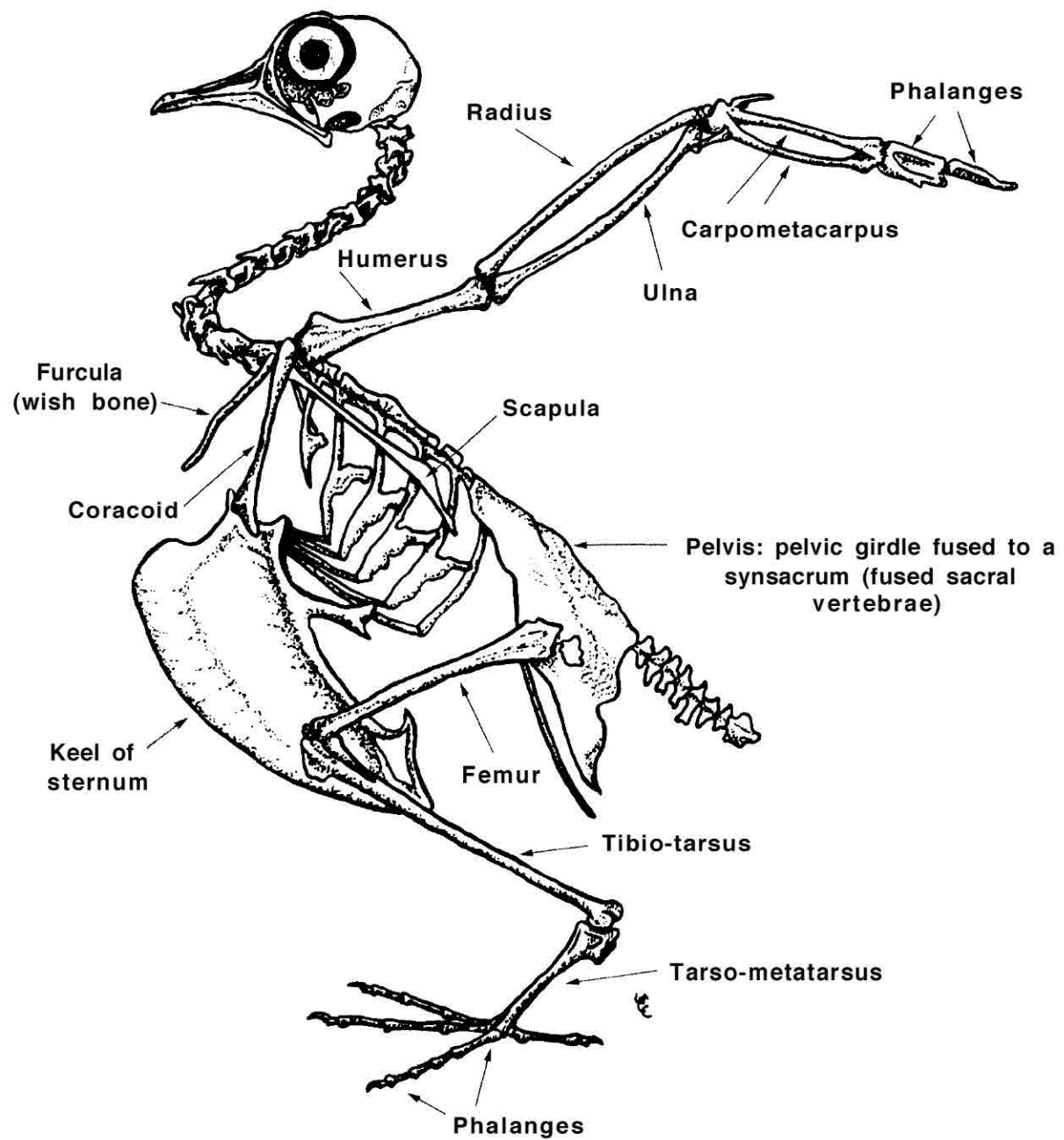
Figure 9.9. Skeleton of the rock dove (*Columba livia*).
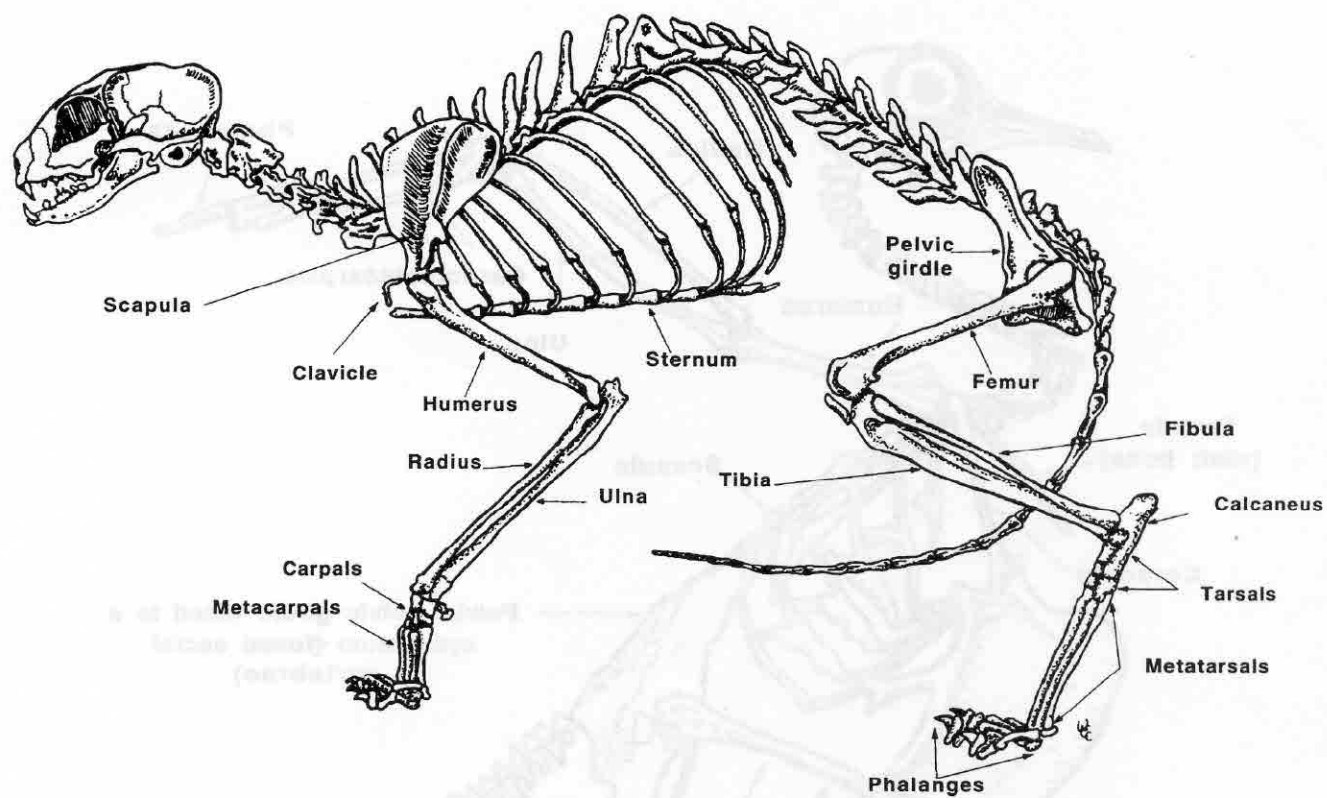
Figure 9.10. Skeleton of the common cat (*Felis domesticus*).
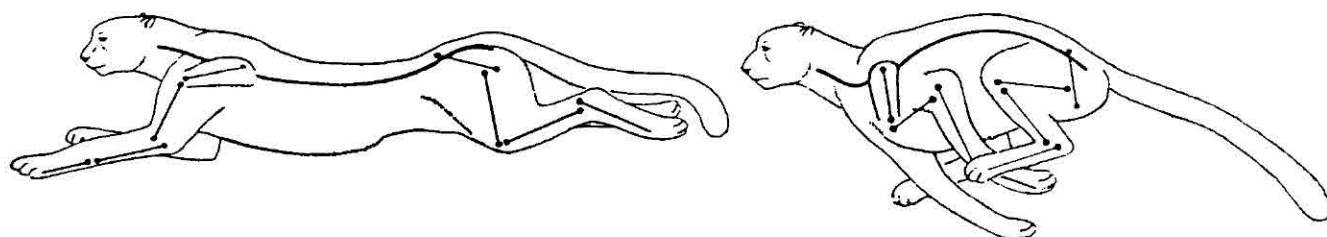
Figure 9.11. Diagrams of a running cat with its back extended and flexed. Lines show the spine and levers in the pectoral and pelvic girdles and legs (Hildebrand, 1974).
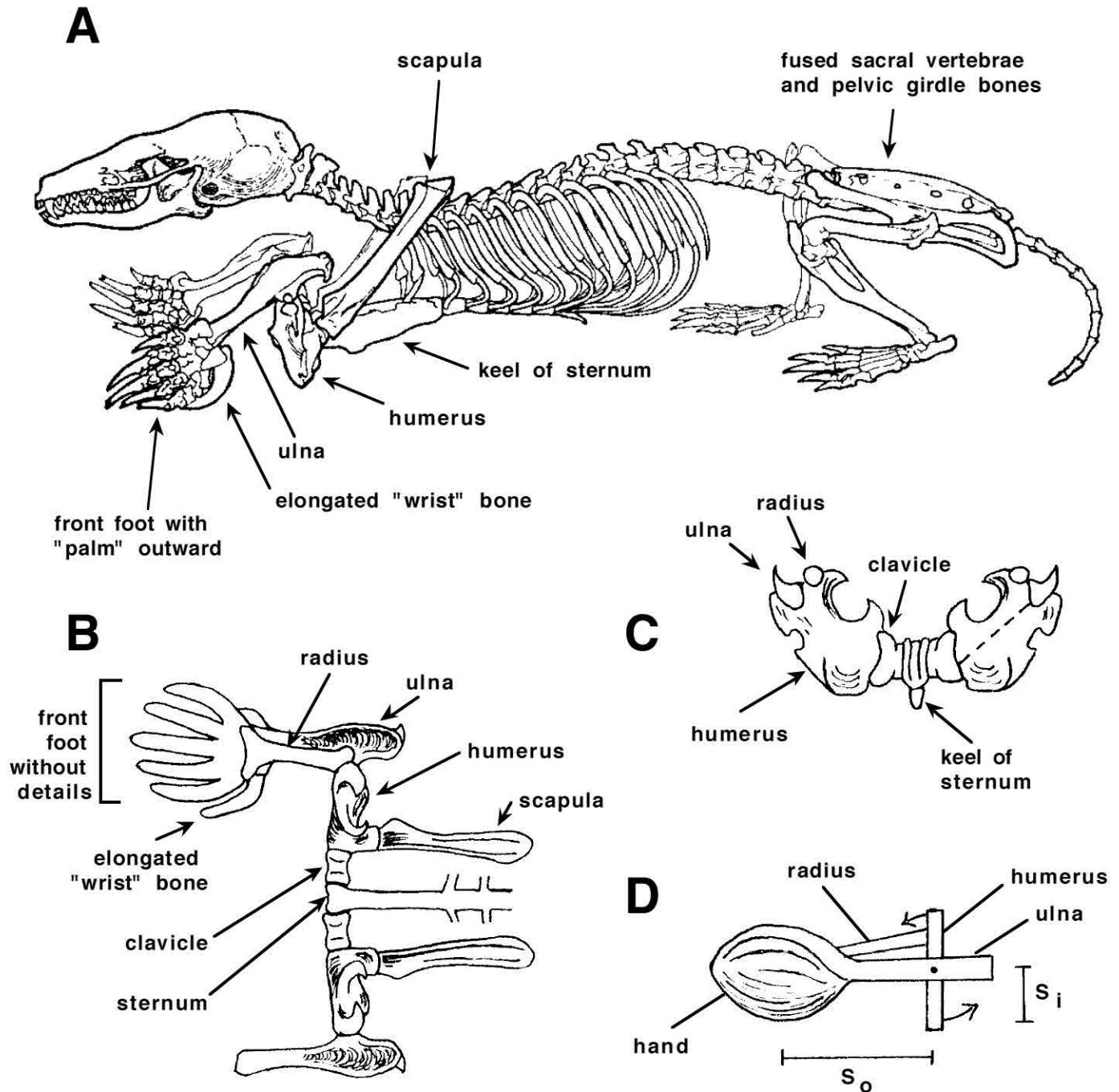
Figure 9.12. Skeleton of the mole (*Talpa europea*). A: entire skeleton, B: dorsal view of the pectoral girdle and articulated bones below the spinal column; both the ulna and radius are shown attached to the right humerus while only the ulna is shown on the left humerus, C: anterior view of the pectoral girdle, humeri and cross sections of radii and ulnae at their articulation sites on the humeri. The broken line is the axis of rotation of the humerus. D: Simplified diagram of the hand-arm lever system used in the power stroke for digging. The center of the out-force is the center of the hand.

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** Included in lab text.

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VERTEBRATE BIOMECHANICS WORKSHEET

Name: _____   **Section:** _____

Application of Biomechanical Principles

Use the diagrams in Figure 9.4 to answer some of these questions.

1.   a.   Which class of lever <u>always</u> has a mechanical advantage less than 1? _____

        b.   Which class of lever is formed by your hand and forearm together rotating on the elbow joint by the contraction of the biceps muscle group (see Figure 9.1)? _____

              Your arm has, in addition to the biceps which inserts on the radius, another forearm flexor muscle, the brachialis, which inserts on the ulna. Both flexors have insertions at approximately the same distance (S_i) from the elbow joint. You can approximate the insertion location of the biceps on the radius by holding your forearm and hand with the palm up perpendicular to your body, and feeling the tendon of the biceps above the elbow (with your other hand).

        c.   Use a meter stick to estimate S_i and S_o on your body for the lever described in part b. Assume F_o is exerted by the palm of your hand.

              $S_i = $ _____ $S_o = $ _____

        d.   How much force must your forearm flexor muscles exert for you to hold a 50 N ($\approx$ 11 lb). weight in your hand with your forearm perpendicular to your body? (Disregard the weight of your forearm and hand.)

2.   a.   Which class of lever <u>always</u> has a mechanical advantage greater than 1? _____

        b.   Stand on the front of your feet with your heels lifted above the floor. Regard each foot as a lever.

            1.   What is the weight (resistance force) being supported by each foot? _____

            2.   Where is the resistance force applied to the foot? _____

            3.   Where is the fulcrum of the foot lever? _____

            4.   Where is F_i applied to the foot lever? _____

            5.   Which class of lever is your foot under these conditions? _____

NOTES ON BIOMECHANICS

How Animals Run

MILTON HILDEBRAND · May 1960

A man (but not necessarily you or I!) can run 220 yards at the rate of 22.3 miles per hour, and a mile at 15.1 miles per hour. The cheetah, however, can sprint at an estimated 70 miles per hour. And the horse has been known to maintain a speed of 15 miles per hour not just for one mile but for 35 miles.

Other animals are capable of spectacular demonstrations of speed and endurance. Jack rabbits have been clocked at 40 miles per hour. The Mongolian ass is reported to have run 16 miles at the impressive rate of 30 miles per hour. Antelopes apparently enjoy running beside a moving vehicle; they have been reliably timed at 60 miles per hour. The camel has been known to travel 115 miles in 12 hours. Nearly all carnivorous mammals are good runners: the whippet can run 34 miles per hour; the coyote, 43 miles per hour; the red fox, 45 miles per hour. One red fox, running before hounds, covered 150 miles in a day and a half. A fox terrier rewarded with candy turned a treadmill at the rate of 5,000 feet per hour for 17 hours.

I have been attracted by such performances as these to undertake an investigation of how the living running-machine works. The subject has not been thoroughly explored. One study was undertaken by the American photographer Eadweard Muybridge in 1872. Working before the motion-picture camera was invented, Muybridge set up a battery of still cameras to make photographs in rapid sequence. His pictures are still standard references. A. Brazier Howell's work on speed in mammals and Sir James Gray's studies on posture and movement are well known to zoologists. Many investigators have added to our knowledge of the anatomy of running vertebrates, but the analysis of function has for the most part been limited to deductions

from skeletons and muscles. The movements of the running animal are so fast and so complex that they cannot be analyzed by the unaided eye.

In my study I have related comparative anatomy to the analysis of motion pictures of animals in action. The method is simple: Successive frames of the motion picture are projected onto tracing paper, where the movements of the parts of the body with respect to one another and to the ground can be analyzed. The main problem is to get pictures from the side of animals running at top speed over open ground. With an electric camera that exposes 200 frames per second I have succeeded in photographing the movements of a cheetah that had been trained by John Hamlet of Ocala, Fla., to chase a paper bag in an enclosure 65 yards long. However, the animal never demonstrated its top speed, but merely loped along at about 35 miles per hour. I have used the same
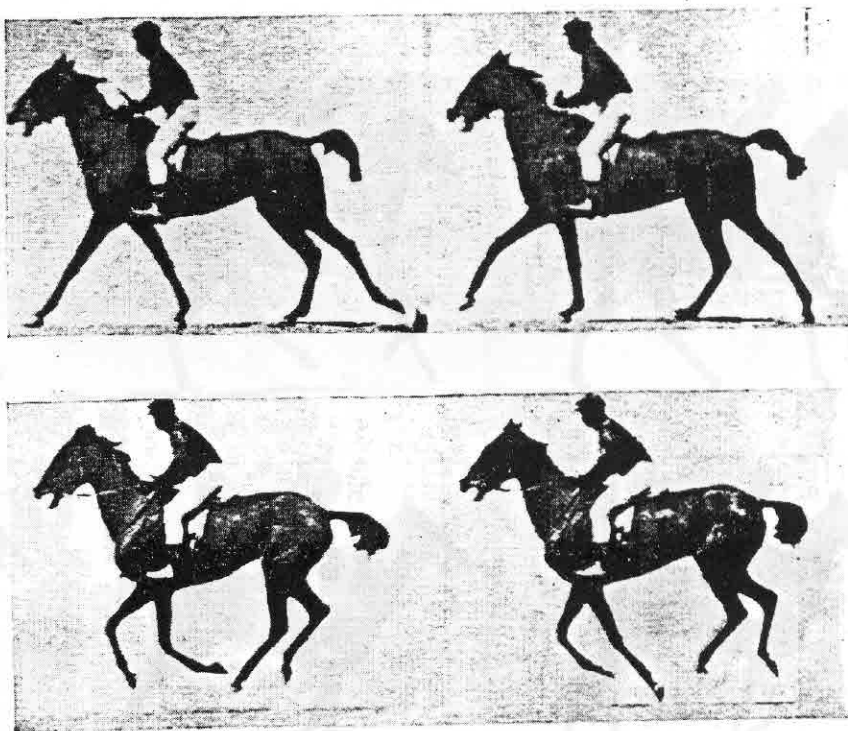
STRIDE OF A CANTERING HORSE is shown in these photographs from Eadweard Muybridge's *The Horse in Motion*, published in 1878. The sequence runs right to left across the

camera to make pictures of horses running on race tracks, and I am presently collecting motion-picture sequences of other running animals from commercial and private sources.

All cursorial animals (those that can run far, fast and easily) have evolved from good walkers, and in doing so have gained important selective advantages. They are able to forage over wide areas. A pack of African hunting dogs, for example, can range over 1,500 square miles; the American mountain lion works a circuit some 100 miles long; individual arctic foxes have on occasion wandered 800 miles. Cursorial animals can seek new sources of food and water when their usual supplies fail. The camel moves from oasis to oasis, and in years of drought the big-game animals of Africa travel impressive distances. The mobility of cursorial animals enables them to overcome seasonal variations in climate or in food supply. Some herds of caribou migrate 1,600 miles each year. According to their habit, the predators among the cursorial animals exploit superior speed, relay tactics, relentless endurance or surprise to overtake their prey. The prey species are commonly as swift as their pursuers, but sometimes they have superior endurance or agility.

Speed and endurance are the capacities that characterize all cursorial vertebrates. But one could not make a definitive list of the cursorial species without deciding quite arbitrarily how fast is fast and how far is far. Even then the list would be incomplete, because there are reliable data on speed for only a few animals; in most cases authors quote authors who cite the guesses of laymen. Many cursors are extinct. On the basis of fossils, however, we can surmise that many dinosaurs were excellent runners; that some extinct rhinoceroses, having had long and slender legs, were very fast; and that certain extinct South American grazing animals, having evolved a horselike form, probably had horselike speed.

In order to run, an animal must overcome the inertia of its body and set it into motion; it must overcome the inertia of its legs with every reversal in the direction of their travel; it must compensate for forces of deceleration, including the action of the ground against its descending feet. A full cycle of motion is called a stride. Speed is the product of length of stride times rate of stride. The giraffe achieves a moderate speed with a long stride and a slow rate of stride; the wart hog matches this speed with a short stride and a rapid rate. High speed requires that long strides be taken at a rapid rate, and endurance requires that speed be sustained with economy of effort.

Although longer legs take longer strides, speed is not increased simply by the enlargement of the animal. A larger animal is likely to have a lower rate of stride. Natural selection produced fast runners by making their legs long in relation to other parts of the body. In cursorial animals the effective length of the leg—the part that contributes to length of stride—is especially enhanced. The segments of the leg that are away from the body (the foot, shank and forearm) are elongated with respect to the segments close to the body (the thigh and upper arm). In this evolutionary lengthening process the bones equivalent to the human palm and instep have become the most elongated.

Man's foot does not contribute to the length of his leg, except when he rises on his toes. The bear, the opossum, the raccoon and most other vertebrates that walk but seldom run have similar plantigrade ("sole-walking") feet. Carnivo-

top row and continues across the bottom row. With these and similar photographs Muybridge settled the controversy of whether or not a horse "even at the height of his speed [has] all four of his feet . . . simultaneously free from contact with the ground."
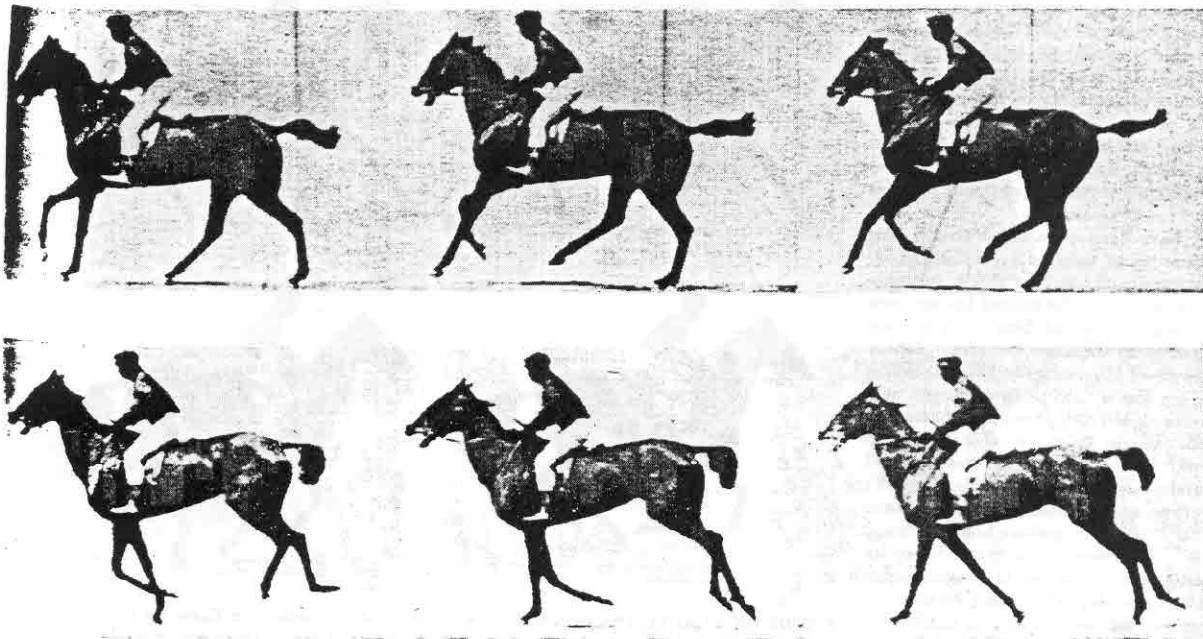
rous mammals, birds, running dinosaurs and some extinct hoofed mammals, on the other hand, stand on what corresponds to the ball of the human foot; these animals have digitigrade ("finger-walking") feet. Other hoofed mammals owe an even further increase in the effective length of their legs to their unguligrade ("hoof-walking") posture, resembling that of a ballet dancer standing on the tips of her toes. Where foot posture and limb proportions have been modified for the cursorial habit, the increased length and slenderness of the leg is striking [see illustration].

The effective length of the front limb of many runners is also increased by the modification of the structure and function of the shoulder. The shoulder joint of amphibians, reptiles and birds is virtually immobilized by the collarbone, which runs from the breast bone to each shoulder blade, and by a second bone,

the coracoid bone. Because mammals do not have a coracoid bone their shoulder blade has some freedom of movement. In the carnivores this freedom is increased by the reduction of the collarbone to a vestige; in the ungulates the collarbone is eliminated. In both carnivores and ungulates the shoulder blade is oriented so that it lies against the side of a narrow but deep chest rather than against the back of a broad but shallow chest, as it does in man. Thus mounted, the shoulder blade pivots from a point about midway in its length, and the shoulder joint at its lower end is free to move forward and backward with the swing of the leg. The exact motion is exceedingly difficult to ascertain in a running animal, but I have found that it adds about 4.5 inches to the stride of the walking cheetah.

The supple spine of the cat and the dog increases the length of stride of these animals still further. The body of such an animal is several inches longer

when the back is extended than when it is flexed. By extending and flexing its back as its legs swing back and forth the animal adds the increase in its body length to its stride. Timing is important in this maneuver. If the animal were to extend its back while its body was in mid-air, its hindquarters would move backward as its forequarters moved forward, with no net addition to the forward motion of the center of mass of its body. In actuality the running animal extends its back only when its hind feet are pushing against the ground. The cheetah executes this maneuver so adeptly that it could run about six miles per hour without any legs.

With the extra rotation of its hip and shoulder girdles and the measuring-worm action of its back, the legs of the running cursor swing through longer arcs, reaching out farther forward and backward and striking and leaving the ground at a more acute angle than they would if the back were rigid. This clear-
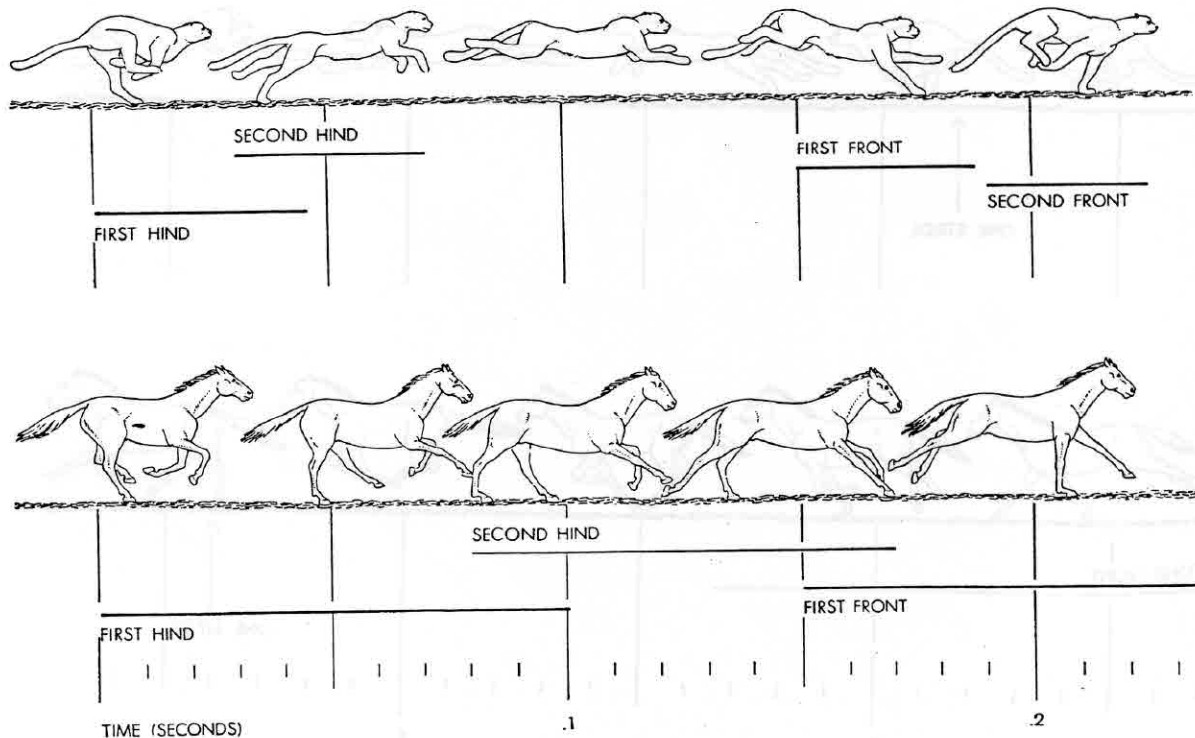
STRIDES OF THE CHEETAH AND THE HORSE in full gallop are contrasted in these illustrations. The sequence and duration of their footfalls, indicated by the horizontal lines under each animal, relate to the time-scale at bottom, which is calibrated in 10ths of a second. The cheetah has two unsupported periods, which account for about half its stride; the horse has one unsupported period,

ly increases stride length, but it also aggravates a problem. The body of the animal tends to rise when its shoulders and hips pass over its feet, and tends to fall when its feet extend to the front or rear. Carnivores offset this bobbing motion by flexing their ankles and wrists, thus shortening their legs. Ungulates do the same by sharply flexing the fetlock joint at the moment that the body passes over the vertical leg. The cheetah, a long-striding back-flexer, supplements its wrist-flexing by slipping its shoulder blade up its ribs about an inch, and thus achieves a smooth forward motion.

Since running is in actuality a series of jumps, the length of the jump must be reckoned as another important increment in the length of the stride. Hoofed runners have one major unsupported period, or jump, in each stride: when the legs are gathered beneath the body. The galloping carnivore has two major unsupported periods: when the back is flexed, and again when it is extended. In the horse all of these anatomical and functional adaptations combine to produce a 23-foot stride. The cheetah, although smaller, has a stride of the same length.

Fast runners must take their long strides rapidly. The race horse completes about 2.5 strides per second and the cheetah at least 3.5. It is plain that the higher the rate of stride, the faster the runner must contract its muscles. One might infer that cursorial animals as a group would have evolved the ability to contract their muscles faster than other animals. Within limits that is true, but there is a general principle limiting the rate at which a muscle can contract. Assuming a constant load on the muscle fibers, the rate of contraction varies inversely with any of the muscle's linear dimensions; the larger muscle therefore contracts more slowly. That is why an animal with a larger body has a slower rate of stride and so loses the advantage of its longer length of stride.

The familiar mechanical principle of gear ratio underlies the fast runner's more effective use of its trim musculature. In the linkage of muscle and bone the gear ratio is equal to the distance between the pivot of the motion (the shoulder joint, for example) and the point at which the motion is applied (the foot) divided by the perpendicular distance between the pivot and the point at which the muscle is attached to the bone. Cursorial animals not only have longer legs; their actuating muscles are also attached to the bone closer to the pivot of motion. Their high-gear muscles, in other words, have short lever-arms, and this increases the gear ratio still further. In comparison, the anatomy of walking animals gives them considerably lower gear-ratios; digging and swimming animals have still lower gear ratios.

But while high gears enable an automobile to reach higher speed, they do

ONE STRIDE

SECOND FRONT

ONE STRIDE

.3   .4
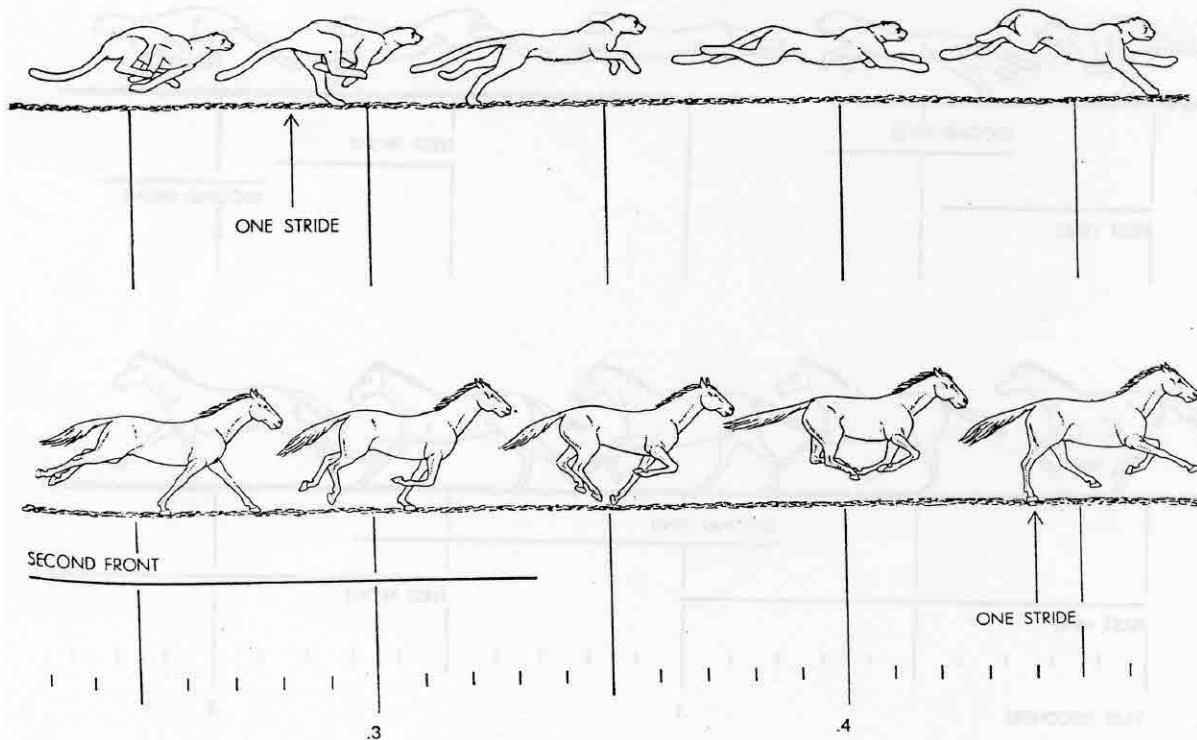
which accounts for about a quarter of its stride. Although both the cheetah and the horse cover about 23 feet per stride, the cheetah attains speeds on the order of 70 miles per hour, to the horse's 43, because it takes about 3.5 strides to the horse's 2.5. The size of the horse has been reduced disproportionately in these drawings for the sake of uniformity in the stride-lines and time-scale.
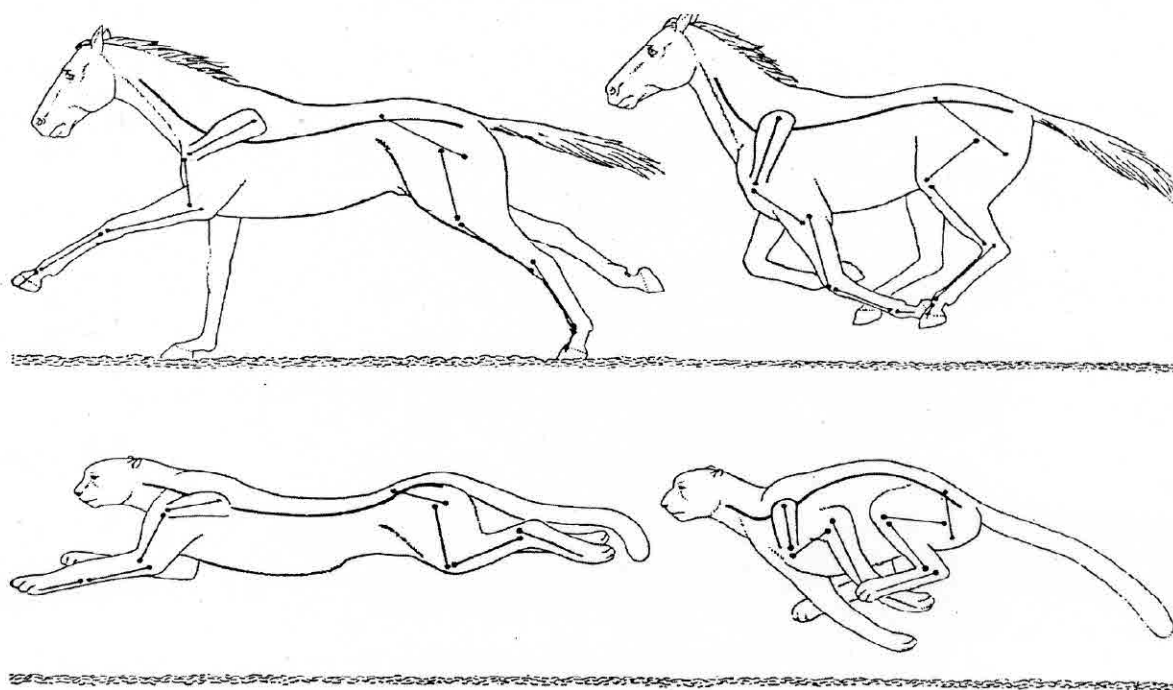
SWIVELING SHOULDER BLADES of the horse and the cheetah add several inches to their stride length. The faster cheetah gains a further advantage from the flexibility of the spine, which in addi- tion to adding the length of its extension to the animal's stride, adds the speed of its extension to the velocity of its travel. Horse's relatively longer leg partially compensates for its rigid spine.
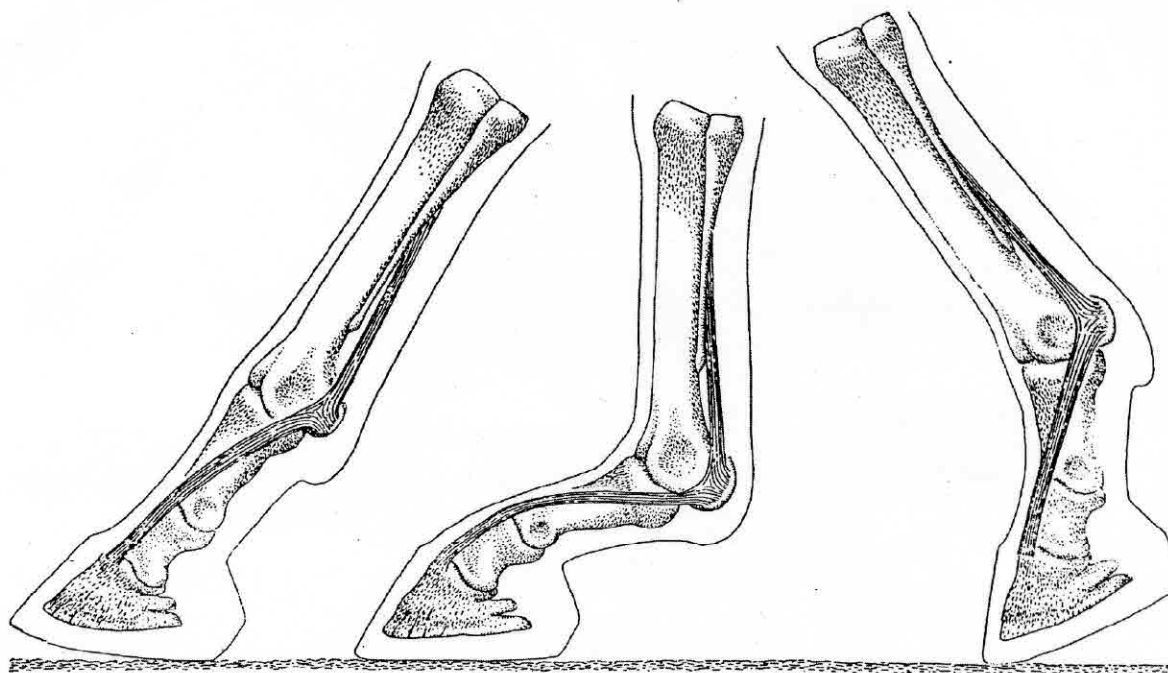
SPRINGING LIGAMENTS in the legs of horses, shown here, and other hoofed runners reduce the need for heavy muscles. Impact of the foot against the ground (*left*) bends the fetlock joint (*mid-* dle) and stretches an elastic ligament (*shown*). that snaps back when the foot leaves the ground (*right*). The springing action at once straightens the foot and gives the leg an upward impetus.

so at the expense of power. The curso-
rial animal pays a similar price, but the
exchange is a good one for several rea-
sons. Running animals do not need great
power: air does not offer much resistance
even when they are moving at top.speed.
Moreover, as the English investigators
J. M. Smith and R. J. G. Savage have
noted, the animal retains some relatively
low-gear muscles. Probably the runner
uses its low-gear muscles for slow mo-
tions, and then shifts to its high-gear
muscles to increase speed.

Since the speed at which a muscle
can contract is limited, the velocity of
the action it controls must be corre-
spondingly limited, even though the
muscle speed is amplified by an opti-
mum gear-ratio. A larger muscle, or
additional muscles, applied to action
around the same joint can produce in-
creased power but not greater speed.
Several men together can lift a greater
weight than one can lift alone, but sev-
eral equally skilled sprinters cannot run
faster together than one of them alone.
The speed of a leg can be increased,
however, if different muscles simultane-
ously move different joints of the leg in
the same direction. The total motion
they produce, which is represented by
the motion of the foot, will then be
greater than the motion produced by
any one muscle working alone. Just as
the total speed of a man walking up an
escalator is the sum of his own speed
plus that of the escalator, so the inde-
pendent velocities of each segment of
the leg combine additively to produce a
higher total velocity.

The trick is to move as many joints
as possible in the same direction at the
same time. The evolution of the cursorial
body has produced just this effect. By
abandoning the flat-footed plantigrade
posture in favor of a digitigrade or un-
guligrade one, the cursorial leg acquired
an extra limb-joint. In effect it gained
still another through the altered func-
tioning of the shoulder blade. The flexi-
ble back of the cursorial carnivore adds
yet another motion to the compound mo-
tion of its legs; the back flexes in such a
way that the chest and pelvis are always
rotating in the direction of the swinging
limbs.

The supple spine of the carnivore
contributes to stride rate by speeding
up the motion of its body as well as of
its legs. The spine is flexed when the
runner's first hind foot strikes the ground,
and by the time its second hind foot
leaves the ground the animal has ex-
tended its spine and thus lengthened its
body. In the brief interval when its hind
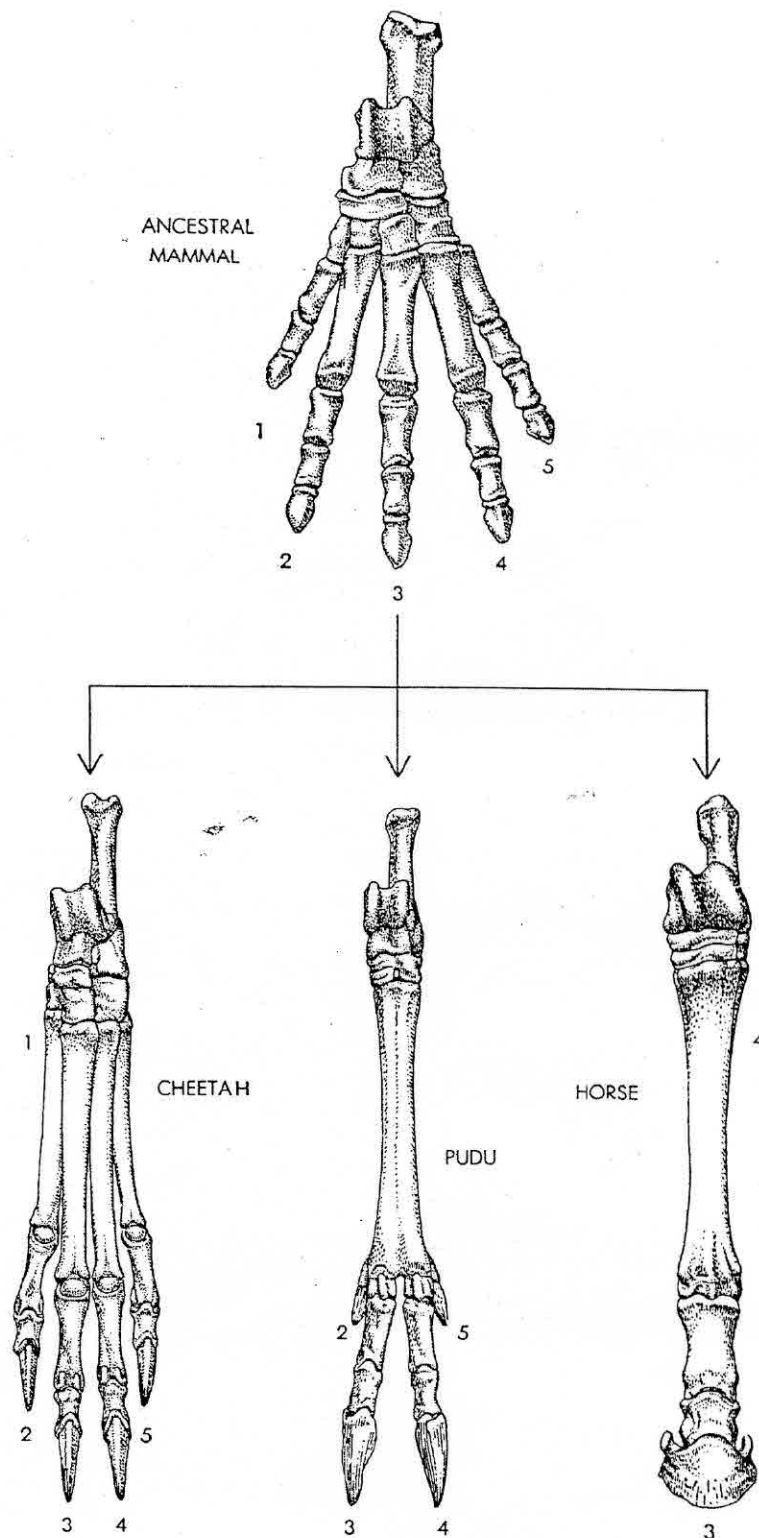
MODERN CURSORIAL FOOT EVOLVED from the broad, five-digited foot of an an-
cestral mammal (top). Lateral digits were lost and metatarsal bones, the longest in the foot,
were further elongated. Resultant foot is lighter and longer. Pudu is a deer of the Andes.

feet are planted, the forequarters, riding on the extending spine, move farther and faster than the hindquarters. Similarly when the front feet are on the ground, the hindquarters move faster than the forequarters. So although the speed that the driving legs can impart to the forequarters or hindquarters is limited by their rate of oscillation, the body as a whole is able to exceed that limit. In a sense the animal moves faster than it runs. For the cheetah the advantage amounts to about two miles per hour—enough to add the margin of success in a close chase.

In addition to the obvious tasks of propelling the animal's body and supporting its weight, the locomotor mus-

cles must raise the body to compensate for the falling that occurs during the unsupported phases of the stride. The load they must raise is proportional to the mass of the body, which is in turn proportional to the cube of any of its linear dimensions. A twofold increase in body length thus increases weight eightfold. The force that a muscle can exert, on the other hand, increases only as the square of its cross section. Thus against an eightfold increase of load, bigger muscles can bring only a fourfold increase of force. As body size increases, the capacity of the muscles to put the body in forward motion and to cause its legs to oscillate cannot quite keep up with the demands placed upon them. These fac-

tors in the nature of muscle explain why the largest animals can neither gallop nor jump, why small runners such as rabbits and foxes can travel as fast as race horses without having marked structural adaptations for speed and why the larger cursorial animals must be highly adapted in order to run at all.

If the bigger runners are to have endurance as well as speed, they must have not only those adaptations that increase the length and rate of their stride, but also adaptations that reduce the load on their locomotor structures and economize the effort of motion. In satisfying this requirement natural selection produced a number of large and fast runners that are able to travel for long distances at somewhat less than their maximum speed. In these animals the mass of the limbs is minimized. The muscles that in other animals draw the limbs toward or away from the midline of the body (the "hand-clapping" muscles in man) are smaller or adapted to moving the legs in the direction of travel, and the muscles that manipulate the digits or rotate the forearm have disappeared. The ulna in the forearm and the fibula in the shank —bones involved in these former motions—are reduced in size. The ulna is retained at the point where it completes the elbow joint, but elsewhere becomes a sliver fused to its neighbor; the fibula is sometimes represented only by a nubbin of bone at the ankle.

The shape of the cursorial limb embodies another load-reducing principle. Since the kinetic energy that must be alternately developed and overcome in oscillating the limb is equal to half the mass times the square of its velocity, the load on muscles causing such motions can be reduced not only by reducing the mass of the faster-moving parts of the limb but also by reducing the velocity of the more massive parts. Accordingly the fleshy parts of the limb are those close to the body, where they do not move so far, and hence not so fast, as the more distant segments. The lower segments, having lost the muscles and bones involved in rotation and in digit manipulation, are relatively light.

The rigor of design imposed by natural selection is especially evident in the feet of cursorial animals. The feet of other animals tend to be broad and pliable; the bones corresponding to those of the human palm and instep are rounded in cross section and well separated. In the foot of the cursorial carnivore, on the other hand, these bones are
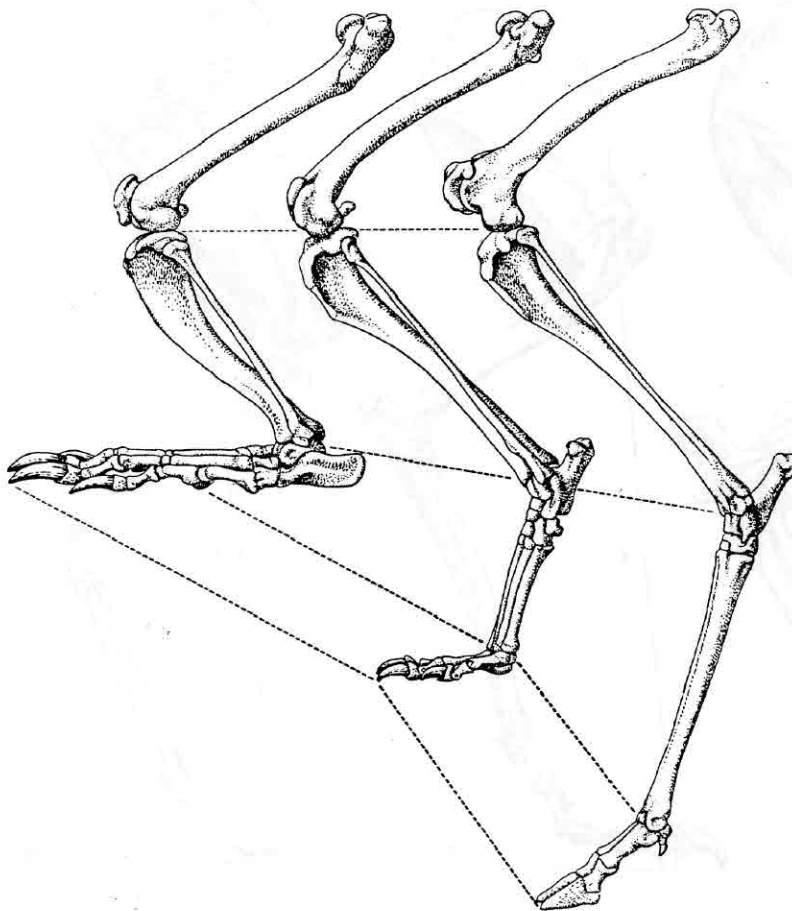
ADAPTATION OF THE LEG FOR SPEED is illustrated by the hind-leg bone of the slow badger (*left*), moderately fast dog (*middle*) and highly adapted deer (*right*). The lengthened metatarsus of the latter two has yielded a longer foot and an altered ankle posture that is better suited to running. The thigh bones of all three animals have been drawn to the same scale to show that the leg segments farthest from the body have elongated the most.

crowded into a compact unit, each bone having a somewhat square cross section. In the ungulates the ratio of strength to weight has been improved still further by reduction of the number of bones in the foot. The ungulates have tended to lose their lateral toes; sometimes the basal elements of the other toes are fused into a single bone. This process gave rise to the cannon bone: the shank of the hoofed mammals [see illustration].

In compensation for the bracing lost as the bones and muscles of their

lower limbs were reduced or eliminated, these animals evolved joints that are modified to function as hinges and allow motion only in the line of travel.

The burden on the muscles of hoofed animals is relieved by an especially elegant mechanism built into the foot. When the hoof of the running animal strikes the ground, the impact bends the fetlock joint and stretches certain long ligaments called the suspensory or springing ligaments [see illustration]. Because the ligaments

are elastic, they snap back as the foot leaves the ground, thereby straightening the joint and giving the leg an upward push. Charles L. Camp of the University of California has found that these built-in pogo-sticks evolved from foot muscles at the time that the animals forsook river valleys for the open plains. The exchange was advantageous, for by means of this and the other adaptations, nature has reconciled the limitations of muscle mechanics with the exacting requirements of speed.
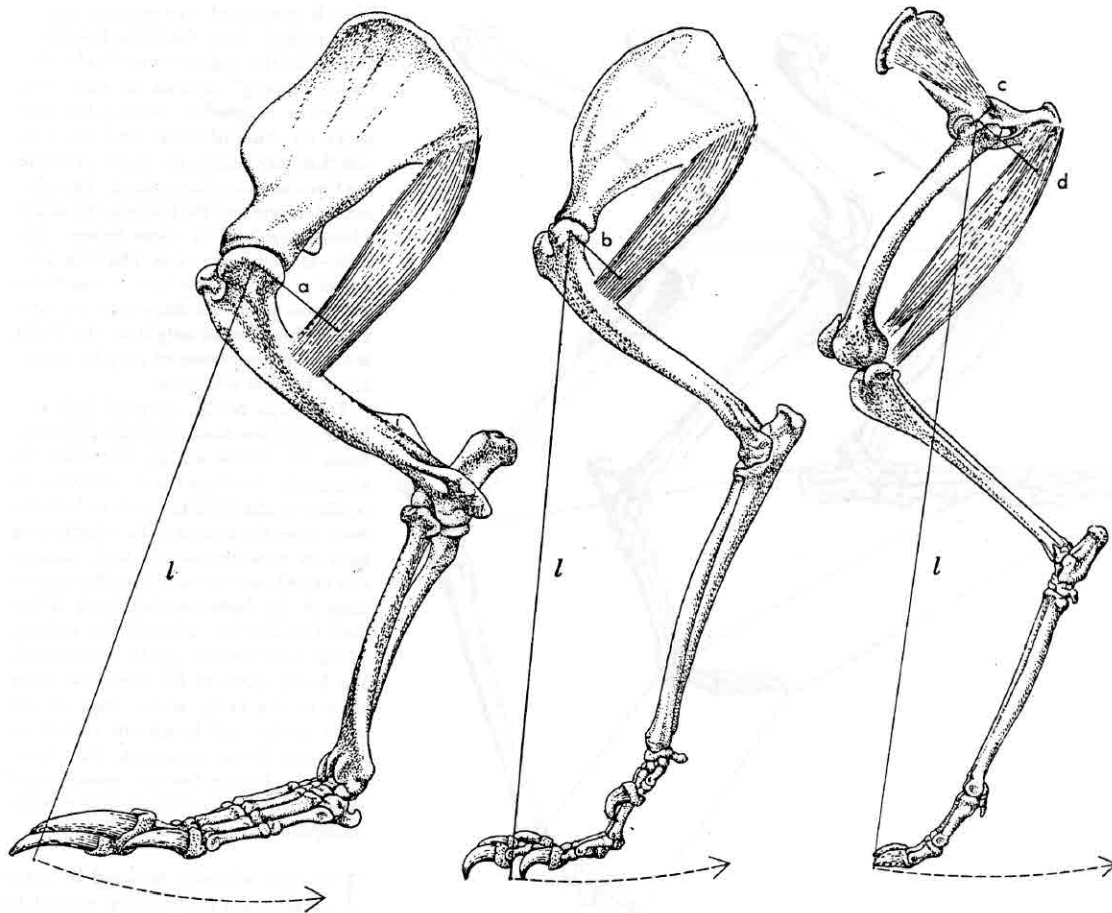
POWER AND SPEED are alternatively achieved in the badger (left) and the cheetah (middle) by placement of the teres major muscle. In the cheetah the small distance (b) between the muscle insertion and the joint it moves yields a higher rate of oscillation than in the badger, in which the distance (a) is greater. The higher oscillation rate, coupled with a longer leg (l), yields a faster stride. In the vicuña (right) the gluteus muscle (c) develops about five times the velocity but only a fifth the force of the larger semimembranosus muscle (d). The animal may use the latter to overcome inertia; the former, for high speed. Legs are not in same scale.

CHAPTER 10 – DIVERSITY OF PHOTOSYNTHETIC PIGMENTS

LABORATORY SYNOPSIS

This laboratory continues your study of protists. In this laboratory you will complete the extraction of photosynthetic pigments from organisms representing three phyla of algae, one plant phylum and from a cyanobacteria (a prokaryote). Then you will use thin-layer chromatography to separate the pigments in each extraction mixture, and identify the characteristic pigments of each phylum. In your discussion/interpretation of the chromatograms, you will consider the principles of thin-layer chromatography and evolutionary relationships among the algae and plants.

The background information provided for this laboratory deals not only with the diversity of pigments found in several groups of photosynthetic organisms, but also discusses the origin and evolution of those groups of organisms. Discussion associated with this lab will be used to further your familiarity with the construction and interpretation of phylogenetic trees.

LABORATORY OBJECTIVES

Conceptual

At the end of this laboratory and the associated laboratory lecture you should

1. know the distinctive photosynthetic pigments of cyanobacteria, plants and the brown, red and green algae.

2. recognize the algal specimens you used and know the protist supergroup and genus for each specimen.

3. be able to describe evolutionary relationships among brown algae, red algae, green algae, plants and the protist supergroups learned in the preceding laboratory exercise.

4. understand endosymbiosis and the proposed origin and evolution of chloroplasts according to the endosymbiotic hypothesis for eukaryotic cell evolution.

5. be able to explain the difference between primary and secondary endosymbiosis.

6. understand and be able to apply the basic concepts and principles of thin-layer chromatography (for example, molecular polarity, adsorption, stationary and mobile phases, solvent systems).

7. know which types of bonds and functional groups cause polarity in pigment molecules.

8. be able to discuss the uses and limitations of pigment analysis in the classification of photosynthetic organisms.

9. know how fluorescence of chlorophyll *a* occurs when a solution of photosynthetic pigments is illuminated.

Procedural

At the end of this laboratory you should

1.   know how to do thin layer chromatography.

READING ASSIGNMENT

In addition to your assigned reading in Campbell et al. (2008), read this chapter's INTRODUCTION, OVERVIEW OF ACTIVITIES, and THIN-LAYER CHROMATOGRAPHY sections.

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

1.   What important reproductive characteristic distinguishes multicellular algae from plants?

2.   Which pigments are found in all organisms that perform oxygen-producing photosynthesis?

3.   According to the endosymbiotic hypothesis for the origin and evolution of eukaryotic cells, what was the precursor of the first chloroplast?

4.   Should the rRNA sequence from a red algal chloroplast be more similar to the rRNA sequence from an amoeba or to the rRNA sequence from a cyanobacterium? How about the rRNA sequence from cytoplasmic ribosomes of the same red alga?

5.   In the thin-layer chromatography system you will use, what is the stationary phase?

6.   a.   What is adsorption?

      b.   What property must a pigment molecule have to adsorb to the stationary phase of the chromatography system used in this lab?

7.   In thin-layer chromatography, what determines how fast a certain kind of molecule will move relative to the movement rate of the mobile phase?

<div align="right">

Paul R. Ecklund
Laurel Hester

Revised June 2010
Mark A. Sarvary

</div>

INTRODUCTION

All algae, all plants and two phyla of bacteria use chlorophyll *a* and other pigments to perform oxygen-evolving photosynthesis. Recall from the previous chapter that the term "algae" applies to eukaryotic, photosynthetic organisms that are not plants and describes a polyphyletic group. Since algae do not form a monophyletic group, Campbell et al. (2008) include algae among the protists in various supergroups, whereas plants continue to be placed in the kingdom Plantae. The distinction between plants, which are multicellular, and the unicellular algae is obvious. However, some types of multicellular algae are larger than many plants and structurally as complex as some simpler plants. The major distinction between complex, multicellular algae and plants is: most algae release zygotes shortly after they are formed, whereas plants retain their zygotes in multicellular female reproductive structures, giving protection and nutrients to developing embryos. This nourishment of multicellular dependent embryos, along with other traits like the presence of apical meristems and use of a desiccation-resistant polymer (sporopollenin) to coat spores, were advantageous in the plant lineage's colonization of land habitats.

In this laboratory exercise, you will analyze the pigments found in algae, plants and a cyanobacterium as part of your investigation of protist phylogeny. Systematists sometimes use the presence of certain molecules (e.g., the desiccation-resistant sporopollenin mentioned above) as characters in their analyses of evolutionary relationships. In several protist clades, the acquisition of chloroplasts led to a rapid diversification of that lineage. The history of these endosymbiotic events can be deduced, in part, from chloroplast pigment composition. Thus, the pigment composition of protist chloroplasts is one character used in evaluating evolutionary relationships among protist groups.

Eukaryotic chloroplasts originated via **endosymbiosis;** the process whereby one cell engulfs another cell which, instead of being digested, becomes an internal symbiont and eventually a plastid. The initial engulfment of a bacterium by another organism is called **primary endosymbiosis**. With regard to chloroplast origin, primary endosymbiosis apparently occurred only once in the eukaryotic lineage when a prokaryotic cyanobacterium became an internal symbiont within the ancestor of the protist Archeaplastida supergroup (thus the name of the group). In the evolution of the endosymbiont to a chloroplast, much of the endosymbiont's genetic material was transferred to the host cell's nucleus, making the endosymbiont dependent on the host cell. However, chloroplasts still contain some DNA and make some proteins on their own ribosomes, which are similar in size and structure to those of prokaryotes.

Ancient members of the cyanobacteria are assumed to have been the endosymbionts that evolved into chloroplasts in the archaeplastida ancestor following primary endosymbiosis. This assumption is based on numerous structural, biochemical and genetic similarities between cyanobacteria and the chloroplasts of photosynthetic eukaryotes. The fossil record verifies that these gram-negative prokaryotes were well established on earth when the first photosynthetic eukaryotic cells originated (about 1.2 billion years ago).

Secondary endosymbiosis occurs when a eukaryote acquires a chloroplast by engulfing another eukaryote. Evidence for secondary endosymbiosis includes the presence of three or four membranes around a chloroplast instead of the more common two (the extra membranes represent the plasma membrane of the engulfed eukaryote and/or the membrane of the host's food vacuole). Sometimes a reduced nucleus can be detected within such a plastid. Evidence suggests that secondary endosymbiosis has occurred multiple times. Thus, information on chloroplast pigments must be combined with evidence as to whether the chloroplasts were acquired via primary or secondary endosymbiosis to correctly interpret evolutionary relationships among algae. Figure 10.1 depicts current understanding of the evolutionary relationships between the groups examined in this lab. Note that the evolutionary relationship

among chloroplasts (e.g., as revealed by analysis of pigments and chloroplast DNA) is not necessarily the same as the evolutionary relationship among their eukaryotic hosts.
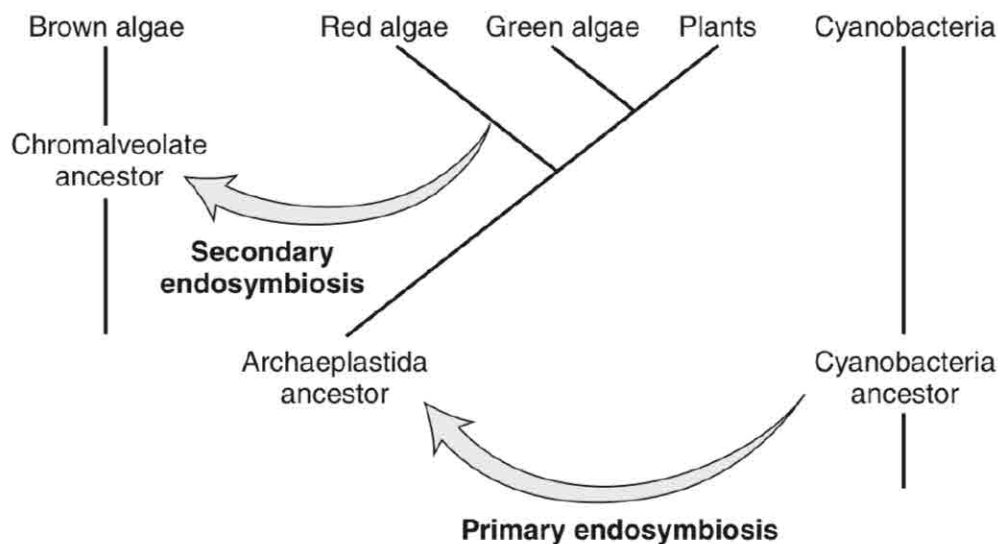
Figure 10.1. Evolutionary relationships among the photosynthetic groups examined in this laboratory exercise are shown. Because exact relationships among protist supergroups are not known, the common ancestor shared by the chromalveolate and archaeplastida groups is not shown. Similarly, while prokaryotes (including the cyanobacteria) and eukaryotes (including algae and plants) do share a common ancestor if you go back far enough in time, the prokaryote/eukaryote split is not depicted here.

Pigments are more easily extracted, visualized and analyzed than is DNA. Additionally, photosynthetic pigments are essential to the ability to perform photosynthesis – a key trait that facilitated adaptive radiation of each eukaryotic group observed in this lab. Figure 10.2 shows the chemical structures of several chloroplast pigments. Molecules of chlorophyll *a* attached to specific proteins function as the catalytic pigment in the two photosystems of all photosynthetic eukaryotes. However, most of the chlorophyll *a* in the photosystems is used to capture photons and/or transfer energy to the catalytic forms of the pigment in the reaction center. All pigments other than chlorophyll *a* assist in absorbing photons to energize the photosystems and are regarded as accessory pigments. For example, chlorophyll *b* and the two forms of chlorophyll *c* are green accessory pigments. These chlorophyll types are found only in certain protist lineages.

The cyanobacterial symbionts from the primary endosymbiotic event apparently evolved with relatively few structural changes into the chloroplasts of the red algae because the cells of cyanobacteria and the chloroplasts of red algae are very similar in structure and pigment composition. Phycobilins (Figure 10.2 C) are the dominant accessory pigments in members of these two groups. The blue-green phycocyanins and the red phycoerythrins consist, respectively, of the pigment molecules phycocyanobilin and phycoerythrobilin covalently bonded to specific proteins. Only red algae and cyanobacteria possess these accessory pigments arranged into complex light harvesting structures called phycobilisomes that are attached to the thylakoid surface.

Green algae apparently diverged from the red algae relatively soon after the archaeplastid lineage originated. Green algae and plant chloroplasts lack phycobilins and instead contain chlorophyll *b*. Recent evidence suggests that the cyanobacterial chloroplast-ancestor contained both phycobilins and chlorophyll *b*. Apparently chlorophyll *b* was lost in the red algae while the biliproteins were lost in the green algal lineage (includes charophyceans and plants). During the 1.2 billion years of evolution since primary endosymbiosis occurred, most cyanobacterial lineages have also lost chlorophyll *b*. However, chlorophyll *b* is retained in prochlorophyte bacteria (e.g., Chlorxybacteria), which appear to be among the prokaryotic descendents of the cyanobacterial chloroplast-ancestor (Tomitani et al., 1999). In this laboratory exercise, you will examine the photosynthetic pigments of *Spirulina*, a modern cyanobacteria popularly used as a nutrient supplement because of its high chlorophyll content.

Chlorophyll *b* is the major photosynthetic accessory pigment in all green algae and plants. It is also found in photosynthetic euglenids (supergroup Excavata, e.g., *Euglena gracilis*) and Chlorachniophytes (supergroup Rhizaria). When biologists observe a trait that is shared by different groups of organisms, they typically hypothesize that the trait evolved only once and is shared because the groups inherited it from a common ancestor. Groups of organisms that share many features are assumed to be more closely related than groups with fewer common features. For example, systematists conclude that green algae and plants are closely related because they have many common features including chloroplasts with a double membrane envelope, starch as the major food reserve and cellulose as the principal cell wall constituent. Recent studies also indicate the presence of many similarities in their DNA sequences. Consequently, systematists conclude that an ancient species of green algae gave rise to plants and place these groups together in the clade Viridiplantae (Campbell et al., 2008).

Most of the features used to define the Viridiplantae clade are not found in photosynthetic euglenids or Chlorachniophytes. Extra membranes surround chloroplasts in these protists, supporting the hypothesis that these chlorophyll *b*-containing photosynthetic protists obtained their chloroplasts via secondary endosymbiosis of a unicellular green algae. Interestingly, many species of euglenids do not have chloroplasts and those with chloroplasts can readily live as heterotrophs in darkness. This lack of dependence on their chloroplasts further supports the idea that these organisms obtained their chloroplasts relatively recently in their evolutionary history. In conclusion, the presence of chlorophyll *b* pigment must be considered in the context of other traits in any analysis of evolutionary relationships.

All algae and plants also possess one or more type of carotenoid pigment (Figure 10.2 D, E and F). Carotenoids include the hydrocarbon carotenes (e.g., beta carotene) and their hydroxyl derivatives, the xanthophylls (e.g., lutein and fucoxanthin). These pigments are yellow, orange and red and absorb light in the blue-green part of the spectrum. Some carotenoids assist in harvesting light for the photosystems. For example, fucoxanthin is the main accessory pigment in diatoms, the golden algae and the brown algae; its relative abundance in their chloroplasts gives these algae their characteristic color. Other carotenoids help protect the photosystems by absorbing blue and near ultra-violet radiation that can damage chlorophyll molecules and other components.

Photosynthetic protists in the Chromalveolata supergroup include diatoms, golden algae and brown algae. Chlorophyll *c* is the accessory chlorophyll and fucoxanthin is the predominant carotenoid in chloroplasts of these protists. The major food reserve in these algae is a polysaccharide called laminarin, and chloroplasts in these groups have four membranes. These shared traits imply a shared evolutionary history among these algal groups; evidence suggests that the common ancestor of the Chromalveolata supergroup acquired its chloroplasts via secondary endosymbiosis of a unicellular red algae, but that chloroplasts were later lost in some of the descendant groups (Sommer et al., 2006).

See Figure 28.2 (p. 577) in Campbell et al. (2008) for another depiction of endosymbiotic relationships. Table 10.1 summarizes the distribution of chloroplast pigments in the various groups described above.

A. Chlorophyll a or b

R$_1$ is CH$_3$ in chlorophyll a
R$_1$ is H–C=O in chlorophyll b
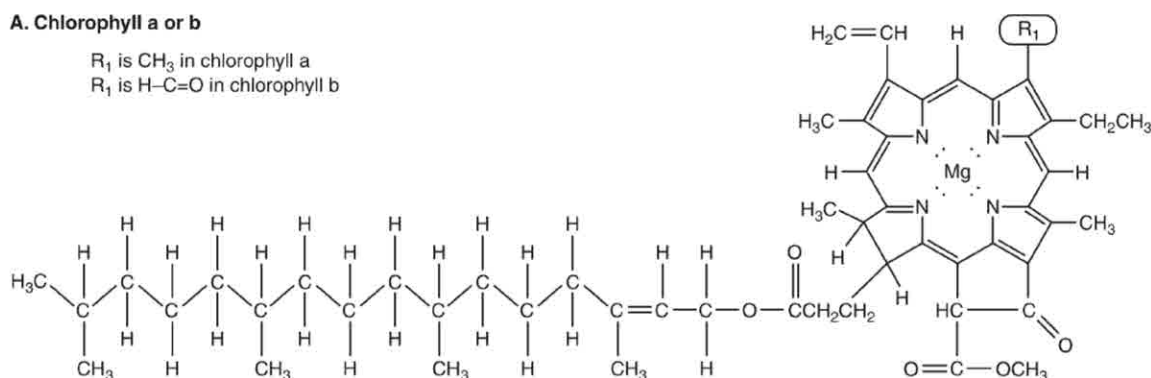
Note: The structure of pheophytin a is not shown – it is identical to chlorophyll a without the Mg^{2+} ion.

B. Chlorophyll c

Chlorophyll c$_1$ is shown
In chlorophyll c$_2$ the circled group is HC=CH$_2$

C. Phycobilins

Phycocyanobilin

Phycoerythrobilin
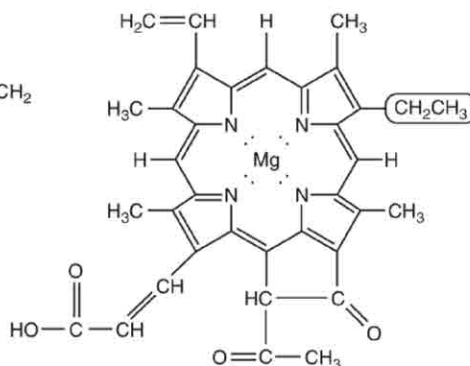
©Hayden-McNeil, LLC
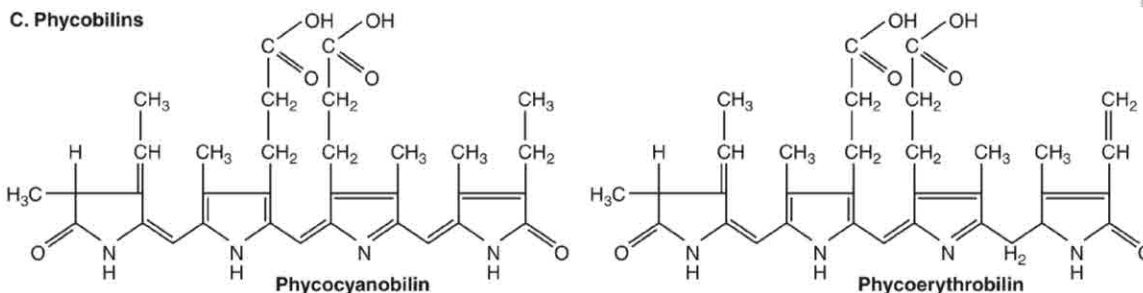
Figure 10.2 (above and next page). Structures of various chloroplast pigments. Lines represent covalent bonds. In the ring diagrams, carbon atoms are at the line junctions without atom symbols. A common feature of the chlorophylls and the phycobilins (A-C) is a structure called a tetrapyrrole which consists of four rings, each composed of four carbons and a nitrogen, linked together. The tetrapyrrole is linear in the phycobilins, but in the chlorophylls it is a ring with a Mg^{2+} ion in the center. Chlorophyll c does not have the long hydrocarbon chain possessed by chlorophylls a and b. In a photosynthetic system, the chlorophylls and phycobilins are conjugated with proteins. D, E and F are carotenoids; E and F are xanthophylls. A common feature of all these pigments is a series of alternating single and double bonds between the atoms. This bonding arrangement enables these molecules to absorb photons and remain chemically stable. Diagrams were adapted from Salisbury and Ross (1969) and South and Whittick (1987).

D. Beta carotene
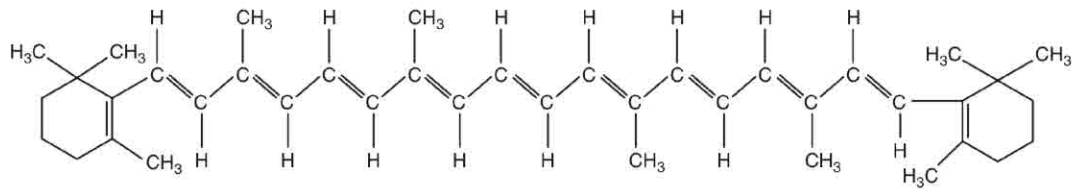
E. Lutein
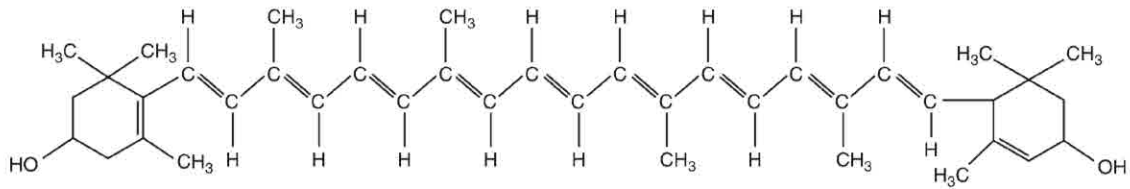
F. Fucoxanthin
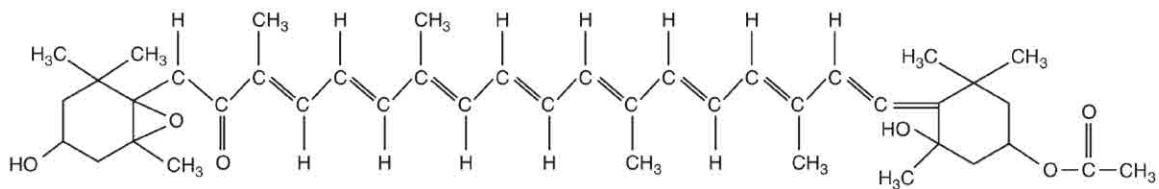
? Mark on Figure 10.1 plausible hypotheses for the evolutionary origin of each photosynthetic pigment discussed above (based on the above discussion of the evolutionary history of photosynthetic pigments). Using a different color, also mark places where pigments may have been lost in certain lineages.

Table 10.1. Pigments in the thylakoid membranes of various groups which perform oxygen-evolving photosynthesis. An X indicates the presence of the pigment(s). Phyla whose names are in bold type are represented in this laboratory.

Photosynthetic groups	Pigments						
	Chlorophylls			Carotenoids			Phycobilins
Phylum (alternative/common name)	a	b	c	Carotenes	Xanthophylls		Phycocyanin & Phycoerythrin
					Fucoxanthin	Others	
Cyanobacteria (blue-green bacteria)	X			X			X
Chloroxybacteria (Prochlorophyta)	X	X		X		X	
Rhodophyta (red algae)	X			X		X	X
Bacillariophyta (diatoms)	X		X	X	X		
Chrysophyta (golden algae)	X		X	X	X		
Phaeophyta (brown algae)	X		X	X	X		
Chlorophyta (green algae)	X	X		X		X	
Anthophyta (flowering plants)	X	X		X		X	

OVERVIEW OF LABORATORY ACTIVITIES

In this laboratory you and your lab mates will concentrate an acetone extract of the chlorophylls and carotenoids from a selected species of each of the algae phyla Rhodophyta, Phaeophyta and Chlorophyta and the plant phylum Anthophyta (flowering plants) and from a Cyanobacteria. You will use a technique called thin-layer chromatography (explained below) to separate and identify the acetone-extracted pigments from each species. Then you will compare the developed chromatograms and look for similarities and differences in the chloroplast pigment composition of the different species.

THIN-LAYER CHROMATOGRAPHY

Chromatography, in its various forms, is used to separate different molecular species from a mixture. The name "chromatography," which means "color writing," was given first to a technique used to separate chloroplast pigments. Now the term applies to numerous techniques used to separate different kinds of molecules, whether they are colored or not. For example, Melvin Calvin and associates employed a form of chromatography to separate and identify the colorless intermediates of the "Calvin cycle" of photosynthesis after they were extracted collectively from a green alga.

All chromatographic systems consist of a stationary phase and a mobile phase (a liquid or gas which moves through the stationary phase). Molecular species are separated by their relative affinities for the two phases. Molecules with a strong affinity for the mobile phase and little attraction for the stationary phase readily move with the mobile phase and experience little hindrance to movement by the stationary phase. On the other hand, molecules that have a weaker affinity for the mobile phase and are strongly attracted to the stationary phase bind reversibly to the latter, inhibiting their rate of movement in the mobile phase.

Thin-layer chromatography (TLC) is often regarded as a microchromatographic technique because the period of time required for separation of different molecular species is relatively short, and relatively small amounts of substances can be separated and detected. TLC entails a process called **adsorption** which is the adhesion of molecules to the surface of a substance called an adsorbent. Adsorption results from the electrostatic attraction between the mobile molecules and the adsorbent material. The stationary phase of our TLC system is the adsorbent, a thin-layer (100 μm in depth) of silica gel (specially prepared silicic acid) bonded to a thin sheet of inert plastic material. The mobile phase is the solvent system consisting of a mixture of toluene and acetone in a volume ratio of 3:2, respectively.

In your use of TLC, you apply a small amount of a solution of extracted pigments as a spot near one end of an adsorbent sheet. Then you put the "spotted" sheet vertically into a test tube containing a small volume of the solvent system and stopper the tube. The solvent system moves up the thin-layer of adsorbent by capillary action. As the solvent system moves up the stationary phase, it picks up the applied pigments and transports them up the adsorbent sheet. The rate at which each chloroplast pigment molecule migrates in the mobile phase depends upon its relative attraction for the stationary phase and for the two different molecular species in the mobile phase.
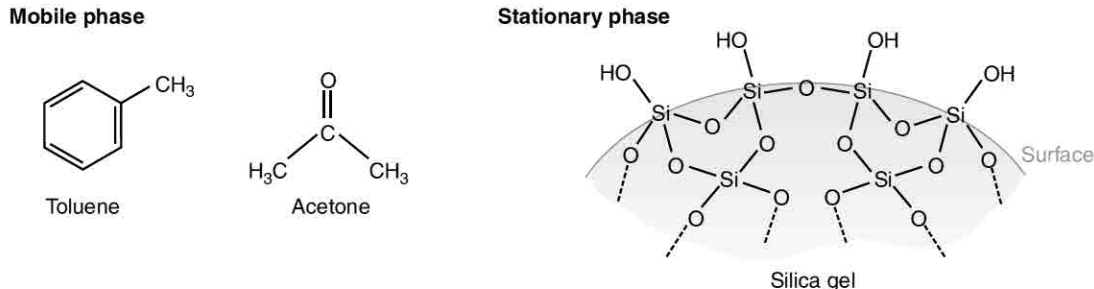
Figure 10.3. Chemical structures of silica (stationary phase), toluene, and acetone (mobile phase).

The stationary phase is a lattice of silica molecules, which are highly polarized (Figure 10.3). Polar regions of pigment molecules are electrostatically attracted to the silica. The mobile phase consists of toluene, which is nonpolar, and acetone, which is polar (Figure 10.3). Toluene attracts nonpolar regions of pigment molecules and acetone has an affinity for both polar and nonpolar parts of the molecules. Since all of these attractive interactions are reversible, the pigment molecules are continually attaching to the adsorbent and being pulled off by the migrating solvent components. The following are empirical generalizations regarding the binding of molecules to the silica gel (stationary phase).
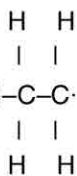
1.   Saturated hydrocarbons (e.g., H–C–C···) are not bound, or are only weakly bound.

       H   H
       | |
     H–C–C···
       | |
       H   H

2.   The binding of unsaturated hydrocarbons increases with the number of double bonds and the number that are in conjugation (alternating single and double C-C bonds; see Figure 10.2 D-F).

3.   Molecular groups substituted for hydrogens in a hydrocarbon increase the binding affinity of the molecule.

       The relative effect of various groups on binding affinity are given below.

$$-O-alkyl^* \; < \; -C=O \; < \; -NH_2 \; < \; -OH \; < \; -COOH$$
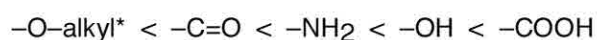
*An alkyl group is a hydrocarbon consisting of one or more carbon atoms saturated with hydrogen atoms.

MORPHOLOGY OF THE SELECTED ALGAL SPECIES

❑   Observe the external morphology of the specimens representing three algae phyla. All three of the specimens are marine algae from the coast of Maine.

       The body of a multicellular alga is called a **thallus**. A complete thallus of any of the three specimens displayed has a basal structure, called a holdfast, to attach it to a firm surface.

❑   Note that each specimen is very flexible, but also very tough with a high resistance to tearing.

?   How are these traits adaptive for these organisms in their natural environment?

SUPERGROUP ARCHAEPLASTIDA; PHYLUM RHODOPHYTA (Red Algae): *Porphyra* sp.

       The thallus is a thin sheet composed of only two cell layers. The specimen may appear more brown than red. The color of the thallus depends on the ratio of its pigments. *Porphyra* exhibits a wide range in color depending on the light intensity of its environment. Organisms growing in shallow water with high light intensity may be dull green. As the water depth of its environment increases (and light intensity decreases) *Porphyra's* phycoerythrin content increases, and it becomes more reddish.

       Porphyra is used as a human food. The Japanese have farmed seabeds of this red alga for many years. Dried sheets of *Porphyra* thalli are called **nori**, which is used in various soups and sauces and for the wrapping of sushi. You may take and taste a small piece of nori.

SUPERGROUP ARCHAEPLASTIDA; PHYLUM CHLOROPHYTA (Green Algae): *Ulva lactuca*

       The thallus is very similar to that of *Porphyra*–a thin sheet of only two cell layers. The common name for *Ulva* is "sea lettuce."

SUPERGROUP CHROMALVEOLATA; PHYLUM PHAEOPHYTA (Brown Algae): *Ascophyllum nodosum*

The thallus is highly branched. Each branch has at least one oblong bladder filled with gas. You may have seen this alga in the seafood section of a market. Fresh seafood is often packed for shipping in *Ascophyllum nodosum* and displayed on it in the store. Your specimen may have reproductive structures, called receptacles, along each branch. Each receptacle is a yellow-green, oblong, pea-sized structure attached by a short stalk. You also may see small, dark red or brownish tufts of the red alga *Polysiphonia lanosa* growing on some of the branches.

ORGANIZATION OF LABORATORY ACTIVITIES

Prior to your arrival in lab, pigments were extracted from the plant, the cyanobacteria and each of the algal specimens by blending each specimen with acetone in an explosion-proof blender. In previous years, students laboriously extracted these pigments by grinding up the specimens using sand and a mortar and pestle. The advantage of the traditional techniques is that it gives one a real appreciation for the toughness of seaweeds, especially the brown algae.

❑ Each group will be assigned to complete the extraction for one of the following specimens:
Red alga (*Porphyra*)
Green alga (*Ulva*)
Brown alga (*Ascophyllum*)
Angiosperm (*Spinacea*)
Cyanobacteria (*Spirulia*)

❑ Each pair/group should obtain the required materials for concentrating the acetone extract from your assigned specimen. Containers of laboratory petroleum ether and anhydrous sodium sulfate are distributed on the laboratory benches.

REQUIRED MATERIALS FOR EXTRACT CONCENTRATION PROCEDURES

1 10 ml graduated cylinder
1 metal test tube rack
1 labelled test tube (initially contains acetone extract of assigned specimen)
1 50 ml **plastic** beakers (thoroughly dry inside; dry with a paper towel, if necessary)
1 30 ml or 50 ml **glass** beaker (thoroughly dry inside; dry with a paper towel if necessary)
1 eyedropper
1 microcentrifuge tube (small, plastic, tapered test tube)
1 microcentrifuge tube rack
1 marking pen

PROCEDURES FOR CONCENTRATING THE PIGMENT EXTRACT FOR CHROMATOGRAPHY

1. Obtain a tube containing 4 ml of your assigned specimen's acetone extract. Use the 10 ml graduated cylinder to measure 6ml of petroleum ether. Add this to your tube of acetone extract.

2. Pour the contents of your tube into your glass beaker; swirl gently to thoroughly mix the petroleum ether and the acetone extract, then carefully pour the mixture back into your test tube.

3. Observe the separation of the liquid phases. The upper layer is a solution of pigments in petroleum ether with some acetone; the bottom layer is acetone and water, and may also contain some water-soluble pigments. Chlorophyll stays in the petroleum ether phase. Layer volumes may differ among specimens due to the different amounts of water in the specimens.

Special Procedure for Extract from *Porphyra* (Red Algae)

The extract from *Porphyra* yields a green upper layer consisting of chlorophylls and carotenoids in a mixture of petroleum ether and acetone and a lower layer, which may be pink, red, purple or blue, consisting of phycoerythrin/phycocyanin in a mixture of acetone and water.

Allow your lab instructor and lab mates to see these two colored layers before you do step 4.

Continue with steps 4-8, but **do not discard the colored acetone-water layer in the test tube (step 9).** Give the test tube containing the acetone-water layer to your lab instructor for a demonstration.

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4. Allow the layers to separate for 3-4 minutes, then use an eyedropper to <u>carefully</u> remove most of the top petroleum ether layer from the test tube (<u>leave the bottom 2 mm of the top layer and do not remove any of the lower acetone/water layer</u>). Quickly dispense the petroleum ether into a <u>clean and dry</u> 50 ml plastic beaker.

5. Use the microspatula to add one level scoop of anhydrous sodium sulfate to the petroleum ether in the beaker. Recap the sodium sulfate container immediately after you use it.

NOTE: The anhydrous sodium sulfate will not dissolve; it removes water from the solution.

6. <u>Under the hood</u> gently swirl the beaker at intervals until the volume of petroleum ether has been reduced to about 0.5 ml (just enough to cover the bottom of the beaker). If you inadvertently allow all the petroleum ether to evaporate, add 0.5 ml of petroleum ether to the beaker and gently swirl it to dissolve the pigments.

7. Carefully pour the remaining pigment solution into a microcentrifuge tube and label the tube with a letter indicating the source of the extracted pigments.

        B = brown alga   G = green alga
        R = red alga   P = plant
        C = cyanobacteria

8. Put the microcentrifuge tube in the tube rack, and put a capillary tube in the microcentrifuge tube.

9. Discard the acetone/water layer in the **Hazardous Waste** bottle in the hood (unless you had the red alga, in which case you should give the acetone/water layer to your lab instructor). <u>DO NOT POUR ACETONE DOWN THE DRAIN.</u>

10. Put beakers, eyedroppers and empty uncapped tubes in designated containers in the hood.

CHROMATOGRAPHY PROCEDURES

❏ Work in groups of 4 or 5.

❏ Each group obtains the following items:

 5 large test tubes with stoppers (these tubes must be completely dry inside)
 1 wooden test tube rack for the large tubes
 1 clean 1 ml pipette
 1 pipetting aid
 2 forceps
 2 rulers

❏ Place the test tubes in a test tube rack. Use the 1 ml pipette with a pipetting aid to dispense 0.8 ml of chromatography solvent (toluene/acetone) into each tube. Stopper each tube immediately after the solvent is added, and keep the tube stoppered until a chromatography strip is put in it.

Procedure for Preparing Chromatography Strips

Precaution: The adsorbent surface of the TLC strip must not be scratched, and it must not be touched directly by fingers; skin oils interfere with the movement of the solvent system through the adsorbent. If the adsorbent becomes wet with water, it is ruined!

1.   Place a clean, flat paper towel on your work surface. Use forceps to carefully remove a TLC strip from the desiccating container and recap the container immediately. Lay the strip, dull side up/shiny side down, on the paper towel.

2.   Use a pencil (not a pen) to make 2 faint marks on one long edge of the strip–one at 1.5 cm from the bottom and the other at 3.0 cm from the top edge. (If you must hold down the strip to do this, put a piece of paper between your fingers and the adsorbent.) Near the top edge, write the letter indicating your pigment source (B, G, R, P or C).

3.   Obtain the appropriate pigment extract. Allow the capillary tube in it to fill by capillary action, then place your finger firmly over the upper end of the tube and keep your finger there while you apply extract to the TLC strip to control the flow of the liquid. If a droplet of extract forms on the tip of the capillary tube, gently blot it on a paper towel before you apply extract to the chromatography strip.

4.   Apply a very small spot of extract in the middle of the width of the strip at 1.5 cm from the bottom (see Figure 10.4). Gently touch the lower tip of the capillary tube to the strip so that only a small amount of extract is wicked out. Allow the spot to dry completely (about 10 sec), then apply another spot on top of the first one. Repeat this application procedure 5 times. (You must open the top of the capillary tube to fill it, but remember to keep it closed while applying extract to the strip.)

❏ Each group member "spots" a chromatography strip with a pigment extract from one of the 5 different sources, and develops the chromatogram. Each group will need chromatograms with pigments from the 5 different sources (3 algae, 1 plant, 1 cyanobacteria), so groups must share extracts.

5.   After completing your application of extract to the TLC strip, allow the spot to dry completely before placing the strip in the chromatography tube. If the spot is dry, it will be dull; if it is still wet it will be shiny. You can hasten the drying of the spot by giving it a gentle stream of air from the air jet on the bench top.

Developing the Chromatograms

<u>Precaution</u>: Leave the chromatography tube in the test tube rack when you put the TLC strip in it and during the development of the chromatogram. Movement of the chromatography tube may cause the solvent system to move unevenly up the adsorbent sheet.

1.   Use forceps to carefully hold the top of the TLC strip and place the strip into the chromatography tube. Then <u>gently</u> stopper the tube.

2.   Observe the movement and separation of pigments as the solvent front moves up the TLC strips. Remove the strip from the tube when the solvent front is 3 cm from the top of the strip. Place the chromatogram on a paper towel to dry.

3.   Discard the TLC solvent in the special waste container in the hood. <u>Do not wash or rinse the chromatography tubes</u>; place them upside down on the pegs of the rack in the hood. Put the stoppers in the labeled container.

Table 10.2. Characteristics of photosynthetic pigments.

Pigment group	Pigment	Color	Soluble in	Relative position on TLC strip
phycobili-	phycoerythrin	red	water-acetone	not applicable
proteins	phycocyanin	blue-green	water-acetone	not applicable
carotenoids	carotenes	yellow, orange	acetone	very high
	xanthophylls	yellow, orange	acetone	high middle to middle
chlorophylls	chlorophyll a	bluish green	acetone	high
	chlorophyll b	yellow green	acetone	slightly lower than chl. a
	chlorophyll c	light green	acetone	very low
	pheophytin a*	gray	acetone	very high

*Pheophytin a is chlorophyll a without a Mg^{2+} ion (see Figure 10.2).

IDENTIFICATION OF PHOTOSYNTHETIC PIGMENTS ON CHROMATOGRAMS

❑ Sketch the pigment band pattern of each chromatogram your group made on the diagrams in Figure 10.3. Compare your group's chromatograms with those of others, especially if any of the pigment bands are faint or blurry. If one of your group's chromatograms did not develop properly (for example, pigments did not separate into distinct bands), sketch the pigment band pattern of another group's chromatogram that did develop properly.

❑ Use the information in Table 10.2 and from your instructor to identify the pigments on each chromatogram. Label the pigment bands with the names of the pigments on Figure 10.4.

Figure 10.4. Distribution of pigment bands on TLC strips developed in toluene/acetone, 3/2, v/v.

| Spirulina (cyanobacteria) | Ascophyllum (brown algae) | Porphyra (red algae) | Ulva (green algae) | Spinach (plant) |
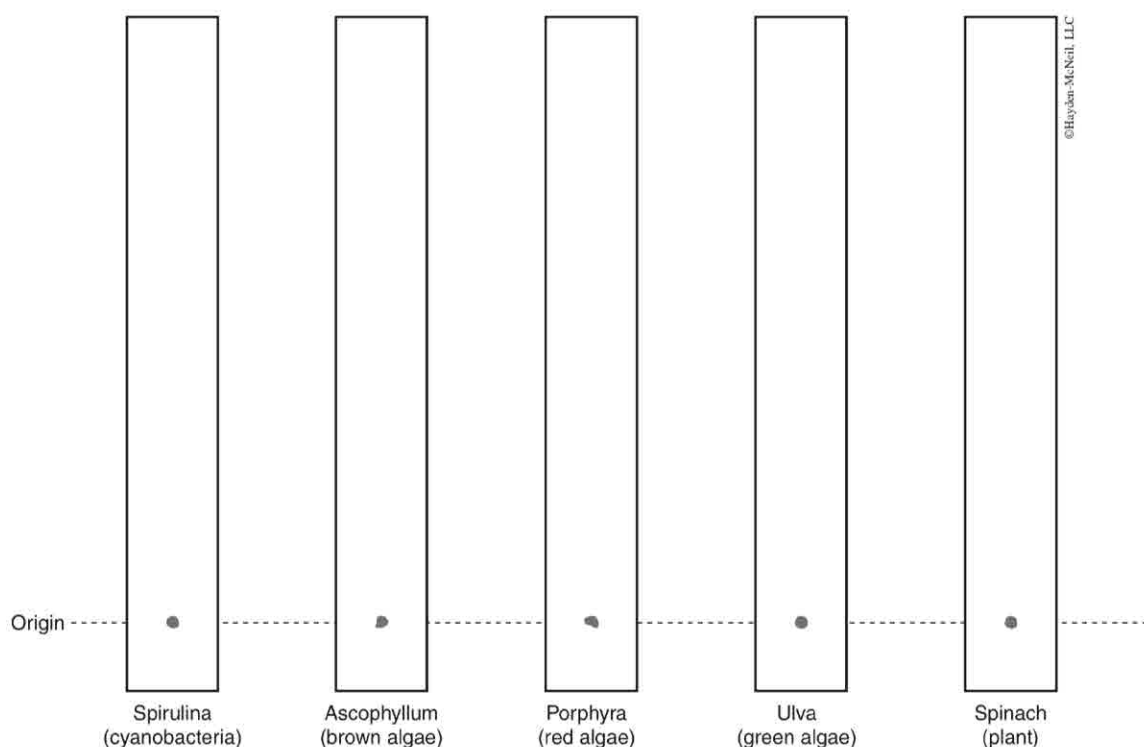
DISCUSSION/INTERPRETATION

❑ Refer to Tables 10.1 and 10.2 and Figure 10.2 for information to answer some of the questions presented.

1. Which pigment(s) is/are on all the chromatograms?

2. A. Which pigment has the lowest affinity for silica?

   B. Explain this very low affinity with respect to the molecules' composition and structure.

3.   A.   Do any of the chromatograms share the same banding pattern of chlorophylls? If so which ones?

      B.   What does this suggest about evolutionary relationship of these groups?

4.   **Accessory pigments** are pigments other than chlorophyll *a* which "capture" photons and transfer the energy to chlorophyll *a*.

      A.   What are the principal accessory pigments of *Porphyra* and all other red algae?

      B.   Which of these accessory pigments were not present on the chromatogram of *Porphyra* pigments, and why were they not present?

5.   Observe the positions of the three chlorophylls on the chromatogram diagrams in Figure 10.4.

      A.   Why did chlorophylls *a* and *b* move farther in the chromatography system than chlorophyll *c* did?

      B.   Why did chlorophyll *a* move farther than chlorophyll *b* in this TLC system?

6.   The color of an alga does not always indicate in which phylum it should be classified. For example, along the coast of the southeastern U.S. there are two different color forms of a common red alga called *Gracillaria*–one red, the other green. Both forms are the same species, but the green form has a mutation which makes it unable to produce phycoerythrin. How could you demonstrate from pigment analyses that a green specimen of *Gracillaria* belongs in the phylum Rhodophyta and not Chlorophyta? (Question adapted from Motten, 1995.)

FLUORESCENCE OF CHLOROPLAST PIGMENTS–DEMONSTRATION

When a green solution of spinach chloroplast pigments in acetone is exposed to rather high intensity light, it appears deep red. The red color is due to the fluorescence of **chlorophyll *a***. All the pigment molecules in solution (chlorophylls *a* and *b* and several carotenoids) absorb radiant energy, but ultimately most of this energy is transferred to chlorophyll *a*, which emits it as photons in the red to far-red portion of the visible spectrum. This same phenomenon occurs within the chloroplast; in fact, there the transfer of energy from accessory pigment molecules to chlorophyll *a* is more efficient because of the highly organized arrangement of pigment molecules within the membrane. In the chloroplast, however, much of the absorbed radiant energy is ultimately transferred to "reaction center molecules" which release it in electrons (instead of photons) transferred to certain electron acceptors in the photosystems. Under light-saturating conditions when the biochemical steps of the light reactions cannot keep pace with the photochemical steps, much of the absorbed radiant energy is released as photons from chlorophyll *a*. Although fluorescence of chlorophyll *a* in chloroplasts and leaves is not readily seen, it can be detected with appropriate instruments.

CLEAN UP PROCEDURES

1.   Pour the solvent from the chromatography tubes into the **Hazardous Waste** bottle in the hood. Do not wash the chromatography tubes; place them upside down on the pegs of the rack in the hood. Put the stoppers in the labeled container.

2.   Pour the pigment extract (if any remains) from the microcentrifuge tubes into the **Hazardous Waste** bottle, then put the microcentrifuge tubes in a designated container also in the hood.

3.   Put capillary tubes in the "broken glass" containers by the sinks.

4.   Put pipettes, tips up, in the "used pipettes" container.

5.   Return all the other equipment to the side bench.

6.   Discard chromatograms in the trashcans if you do not wish to keep them.

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APPENDIX 1
THE METRIC SYSTEM

Paul R. Ecklund

This appendix includes the units of the metric system and their symbols (abbreviations) which are most commonly used in the literature. There is a basic unit of linear measure, mass and capacity; all other units are powers of 10 of the basic unit. Some units have more than one name and symbol.

TYPE OF MEASURE	UNIT		SYMBOL (ABBREVIATION)*
LINEAR	meter	(basic unit)	m
	kilometer	$= 10^3$ meters	km
	decimeter	$= 10^{-1}$ meter	dm
	centimeter	$= 10^{-2}$ meter	cm
	millimeter	$= 10^{-3}$ meter	mm
	micron	$= 10^{-6}$ meter	μ
	micrometer	$= 10^{-6}$ meter	μm
	millimicron	$= 10^{-9}$ meter	mμ
	nanometer	$= 10^{-9}$ meter	nm
	angstrom	$= 10^{-10}$ meter	Å
MASS	gram	(basic unit)	g (sometimes gm)
	metric ton	$= 10^6$ grams	no standard symbol
	kilogram	$= 10^3$ grams	kg
	milligram	$= 10^{-3}$ gram	mg
	microgram	$= 10^{-6}$ gram	μg
CAPACITY	liter	(basic unit)	l
	kiloliter	$= 10^3$ liter	kl
	milliliter	$= 10^{-3}$ liter	ml
	microliter	$= 10^{-6}$ liter	μl
AREA	hectare	$= 10^4$ square meters	ha
	all other units of area are expressed as the square of linear units.		linear unit ^{2} (e.g., m^2 for square meters)
VOLUME	All units of volume are expressed as the cube of linear units.		linear unit ^{3} (e.g., cm^3 for cubic centimeters; some–times the abbreviation cc is used for cubic centimeter(s))
	lambda	$= 10^{-3}$ cm^3 $=1$ mm^3	λ

In many cases units of capacity are used for volume because, for all practical purposes, 1 milliliter = 1 cubic centimeter.

*Note: By convention, periods are never used after metric symbols, and the symbols are not pluralized by adding "s" (e.g., the symbol mm should be used for one or more than one millimeter). Remember this in your laboratory reports.

USING UNITS

Numbers without units are often meaningless. For instance, if someone when asked how far it was to downtown Ithaca from the Cornell campus answered "15" what would that mean? Did they mean: 15 miles? 15 Kilometers? 15 minutes of walking? Units are essential!

Beyond stating the basic unit, choosing which prefix of the unit to use is a matter of convenience. For example, mass can be expressed as grams, milligrams, kilograms, etc. Thus for a single gram: 1 g = 0.001 kg = 1000 mg = 10^{-6} metric tons. Here "1 g" is the most convenient form, whereas expressing just a single gram in terms of metric tons is very odd. On the other hand if we wanted to express the harvest of corn from a field: 2.2 metric tons = 2.2 x 10^6 g = 2,200 kg = 2,200,000,000 mg. Here using metric tons would be appropriate.

LEVEL OF CERTAINTY AND ROUNDING OFF

The level of certainty to which measurements are reported is important. For instance, a measurement that is reported to the nearest meter (i.e. 4 m) would be different from another measurement, also expressed in meters, which is reported to the nearest millimeter (i.e. 4.000 m). In the first case we could assume that the estimate of 4 m is uncertain to plus or minus one meter, so the object measured could actually be within the range of 5-3 meters. In the second case the uncertainty is at the level of a thousandth of a meter, and so the object measured could be taken to be in the range of 4.001-3.999 m. The first case has just one significant digit, whereas the second case has four significant digits. A proper report of a measurement indicates by the use of the correct number of significant digits its level of certainty.

Generally when doing calculations with numbers of limited levels of certainty all digits are retained in any intermediate calculations. It is only the final result of the calculation that is rounded off to the correct level of certainty.

For example: Measurements were made of the mass of seeds found in an area. These measurements were: 1.25 g, 4.68 g, 1.35 g, 2.25 g. How then would the mean seed mass and its standard deviation be reported? Clearly the uncertainty of the measurements is at the range of a hundredth of a gram (i.e. 0.01 g). Thus the answers should be reported to the nearest hundredth of a gram.

In doing the calculation of the mean we get as a result:

(1.25 g + 4.68 g + 1.35 g + 2.25 g)/4 = (9.53 g)/4 = 2.3825 g per seed.

Thus the mean seed mass would be reported as 2.38 g.

For the standard deviation (see Appendix 4 for how to do this calculation, you can try it yourself if you wish) the result of the calculation is 1.59631607146 g. Thus the standard deviation of the seed mass would be reported as 1.60 g.

Notice that in both cases the uncertainty in the last digit (in this case 0.01 g) extends beyond the changes resulting from rounding off. This is typical.

APPENDIX 2
INSTRUMENTATION

Paul R. Ecklund and Jon C. Glase

SPECTROPHOTOMETRY

Photometry is the process of measuring the intensity of light. Spectrophotometry is a technique which uses light of a single wavelength or a very narrow range of wavelengths, and allows one to estimate the amount of a substance in solution by measuring the intensity of light transmitted by the solution. As well as using "light", spectrophotometry, with the appropriate instrumentation, can use wavelengths in the ultraviolet or the infrared portions of the radiation spectrum.

Spectrophotometry depends upon the interaction between matter and light. Electromagnetic radiation, including light, is considered to have a wave motion and to consist of discrete packets of energy called quanta. The energy of a quantum is directly proportional to its frequency of vibration, and inversely proportional to the wavelength.

Every material absorbs some wavelengths of radiant energy. If the absorbed wavelengths are in the visible protion of the spectrum, the material is considered to have color, because the human eye perceives only those wavelengths of light that are not absorbed, but are transmitted or reflected by the material. If no visible wavelengths of light are absorbed, the material appears white or colorless; if nearly all visible wavelengths are absorbed, the material appears black.

The absorption of radiant energy occurs at the molecular level. When a molecule absorbs light energy, it must absorb a **single** and **complete** quantum. The number and distribution of electrons in a molecule determine what size of quantum (what amount of energy) the molecule can absorb. Thus a molecular species is characterized by the wavelengths of light that it can absorb. Because spectrophotometry uses a very narrow range of wavelengths, it can be used to determine the relative amounts of different wavelengths that are absorbed by a substance in solution. A plot of the relative amount of radiation absorbed by a substance as a function of the wavelength of the radiation is called an **absorption spectrum** of the substance.

FUNDAMENTAL PRINCIPLES OF SPECTROPHOTOMETRY

Throughout the following discussion the term "light" is used; however, this term is meant to include the ultraviolet and infrared, as well as the visible portions of the electromagnetic radiation spectrum.

When light of intensity I_o strikes a solution in a transparent container as shown in Figure 1, some of the light (I_s) is scattered or reflected by the container's walls and by suspended particles in solution; some (I_a) is absorbed; and some (I_t) is transmitted. $I_o = I_s + I_a + I_t$.
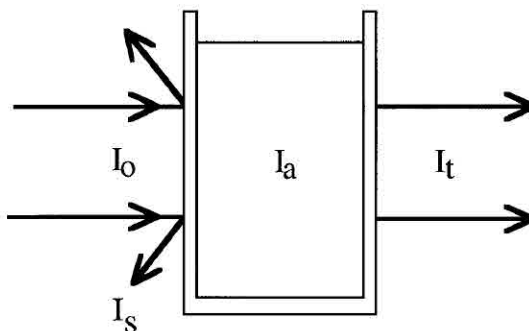
Figure 1. The distribution of incident light by a solution in a transparent container.

Spectrophotometry is based upon the relationship between the amount of light absorbed (I_a) and the concentration of the absorbing substance; however, the instrumentation employed detects only the amount of light transmitted by the solution and its container (I_t). Since light scattering by the container cannot be eliminated, it is kept constant by using containers which have essentially identical optical properties when I_o and I_t are measured. Suspended particles in the solution also contribute to I_s; therefore, accurate determinations of I_a can be obtained only with true solutions.

A **spectrophotometer** is used to measure I_o and I_t. In it the transparent container of solution is subjected to a beam of monochromatic light (a narrow band of wavelengths) and I_t is detected by a photocell. The output of the photocell indicates the magnitude of I_t. The measurement of I_o is accomplished by determining I_t of a container of solvent <u>without the light-absorbing substance dissolved in it</u>. When measuring I_o the instrument is set to record 100% light transmission. In this way the instrument is set to indicate that all of the light passes through the pure solvent, and I_s by the container is "ignored." Under these conditions $I_o = I_t$ because no light-absorbing substance is present; consequently $I_a = 0$. When a container with a solution of the light-absorbing substance is in the light beam of the spectrophotometer, I_t is less than that obtained when pure solvent is used because some light is absorbed by the substance. In this case $I_a = I_o - I_t$. The fraction of the incident light that passes through a light-absorbing solution is called the **transmittance** of that solution and is expressed as:

$$\text{transmittance, } (T) = \frac{I_t}{I_o}$$

Usually T is expressed as a percentage.

Transmittance decreases exponentially with the concentration of the light-absorbing substance; consequently, it is not convenient to use in determining concentrations. However, the **absorbance** (A) of a light- absorbing substance varies directly with its concentration (at least within a certain range of concentrations) and can easily be used to determine an unknown concentration. Absorbance is defined in the following ways:

$$A = -\log T = abc$$

Where a is a constant called the molar extinction coefficient of the light- absorbing substance, b is the length of the light path through the absorbing substance in centimeters, and c is the concentration of the light-absorbing substance expressed in <u>moles per liter</u>. This relationship is valid only if monochromatic light is used and if the nature of the absorbing material remains constant at different concentrations (i.e., molecules of the absorbing material do not associate or dissociate at different concentrations).

It should be noted that absorbance is sometimes called **optical density** or **extinction**; however, the term absorbance is most frequently used.

SPECTROPHOTOMETRIC DETERMINATION OF AN UNKNOWN CONCENTRATION

Generally, the wavelength of light used is one which is <u>maximally</u> absorbed by the substance being studied. This wavelength can be found from an absorption spectrum determined by the investigator or in the literature. Most spectrophotometers have scales showing both percent transmittance and absorbance, so either can be directly measured. Obviously, from the relationship A = abc, if a and b are known, c can be readily determined when A is measured. However, frequently the value of a is not readily obtainable. In this case the unknown concentration of a substance is determined by comparing the absorbance of a solution of unknown concentration to the absorbance of a solution of the same substance whose concentration is known. The following relationships exist for each solution:

$$A_k = a_k b_k c_k$$
$$A_u = a_u b_u c_u$$

where the subscripts k and u indicate the solutions whose concentrations are known or unknown, respectively. Since both solutions contain the same light-absorbing substance and the same solvent, a is the same for both solutions because it is a constant under these conditions. Furthermore, by using the same light path, b is the same for both solutions. Consequently, the different values of A of the two solutions depend only on the different concentrations, and the following relationship exists:

$$\frac{A_k}{A_u} = \frac{c_k}{c_u} \qquad \text{OR} \qquad c_u = \frac{A_u c_k}{A_k}$$

When A_u and A_k are measured, c_u can be determined.

THE SPECTROPHOTOMETER: DESCRIPTION AND USE

All laboratories are equipped with Bausch and Lomb Spectronic 20 spectrophotometers. Figure 2 is a picture of the instrument indicating the external functional components, and Figure 3 shows the optical system of the instrument.

A spectophotometer is an instrument that enables one to direct a beam of monochromatic (one wavelength) light onto a substance (usually a solution) and measure the amount of that light transmitted and absorbed by the substance. The Spectronic 20 is a single beam spectrophotometer with a wavelength range from 340 nanometers (nm) to 950 nm; however, this range of wavelengths is obtainable only by using more than one phototube-filter combination. The monochromatic light beam is produced by directing a beam of mixed wavelengths of light onto a **diffraction grating** which acts as a series of tiny prisms to separate the wavelengths of light. The desired wavelength is selected by the position of the grating, and light of this wavelength is reflected and directed toward the sample holder and measuring phototube (see Figure 3). Actually, a light beam of a single wavelength is not obtainable with the Spectronic 20; the light beam is composed of the selected wavelength and wavelengths as much as 5 nm shorter and longer than that selected.

The spectrophotometer has two scales–a <u>linear</u> scale for **percent transmittance (%T)** and a <u>logarithmic</u> scale for **absorbance (abs)**–which are inversely related. Generally an investigator wants absorbance values, since they are directly related to the concentration of the substance being studied; however, absorbance values above 0.5 cannot be accurately read directly from the spectrophotometer. Percent transmittance values can be read accurately and these can be converted to absorbance values by using Table 1 or by determining the log of the inverse of the transmittance.

Preparation of the Samples

Spectrophotometry involves comparing the absorbance of a "test" solution containing the substance whose concentration is being determined with the absorbance of a reference solution called a "**blank**". A "blank" solution usually has the same composition as a "test" solution <u>except it lacks the substance whose concentration is being determined</u>.

The solution container is a special glass tube called a **cuvette** which has an index mark on its side.

NOTE: Cuvettes require careful handling to prevent scratching them; scratches alter their optical properties. DO NOT do the following: (a) allow the cuvettes to rub against each other, (b) place cuvettes in metal test tube racks, or (c) wash cuvettes with a brush or any abrasive cleanser.

Each sample must meet the following criteria prior to its placement in the spectrophotometer:

1.   The solution is free of bubbles.
2.   The cuvette is at least half full.
3.   The outside of the cuvette is dry and clean (no smudges such as fingerprints).
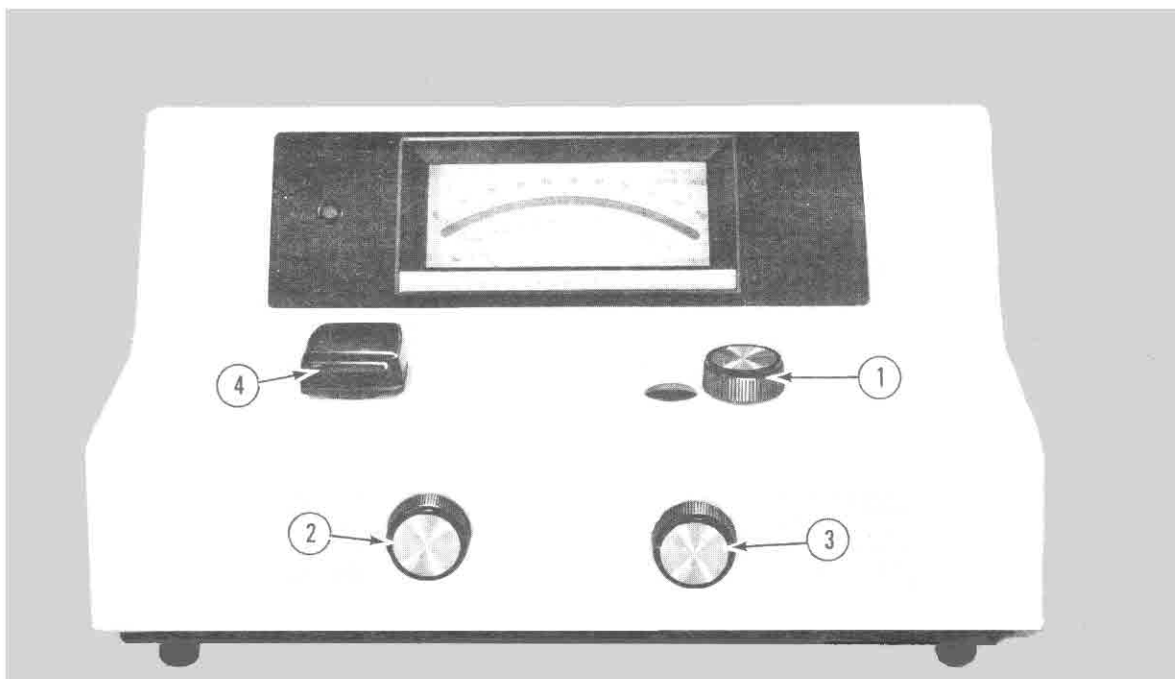
Figure 2. Bausch and Lomb Spectronic 20. (Reprinted with permission of Bausch and Lomb) (1) Wavelength control knob. (2) Power switch/zero control knob. (3) 100%T control knob. (4) Sample holder.
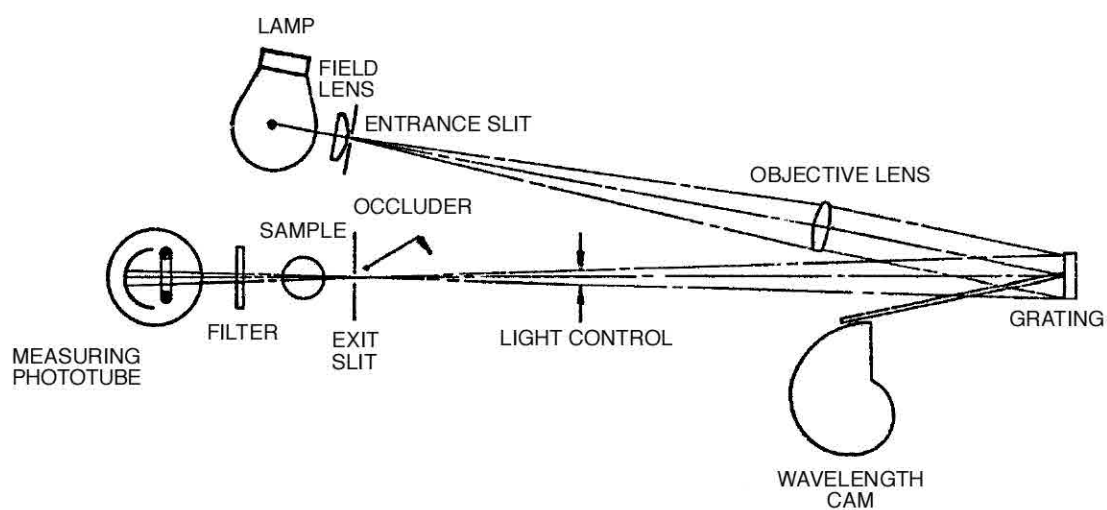
Figure 3. Spectronic 20 Optical System. (Reprinted with permission of Bausch and Lomb)

Measurement of Absorbance of a Solution

Numbers in parentheses correspond to components of the Spectronic 20 as indicated in Figure 2.

1. Turn on the spectrophotometer (2) and allow it to warm up for about 10 minutes.

2. Set the wavelength control (1) to the desired wavelength.

3. Establish that the sample holder (4) is empty, and close the lid.

4. Adjust the **left knob** (2) until the needle indicates 0% transmittance (%T). When the sample holder is empty, an occluder automatically blocks the light path so no light is transmitted through the sample holder (see Figure 3).

5. Place a cuvette with **blank** solution in the sample holder (4); align the index mark on the cuvette with the mark on the holder, and close the lid of the holder.

    NOTE: With any sample, the index mark on the cuvette should be aligned with the mark on the holder.

6. Adjust **right knob** (3) until the needle indicates 100%T. Since the blank solution does not contain the substance whose absorbance is being measured, the instrument is arbitrarily set to indicate that no light is absorbed by the blank (all the light is transmitted); any light absorbed or scattered by the blank and its cuvette is "ignored" by the instrument.

7. Repeat steps 4, 5 and 6 until no adjustment of knob (2) or (3) is required for the needle to indicate 0% and 100%T.

8. Place a cuvette with a "test" solution in the sample holder and close the lid. As soon as the needle stabilizes, read %T to the nearest 1/4 of 1% T.

9. Remove the "test" solution from the sample holder and repeat steps 4, 5, and 6 to make sure the instrument has not drifted from zero or 100%T.

    NOTE: The Spectronic 20 has a tendency to drift from zero and 100%T during an extended period of time; therefore, one must check the instrument for these settings (steps 4, 5 and 6) prior to **each** measurement of a "test" solution.

    The instrument must be reset to zero and 100%T whenever the analytical wavelength is changed.

10. Convert the value of %T obtained from each "test" solution to an absorbance value using Table 1 on page A8. Using the whole %T of your reading, select the correct line of the table. Now, based on the 1/4 of 1%T of your reading, use the column headings (0.0, .25, .50, .75) to select the correct column of absorbance readings for that line of the table. For example, a reading of 62.75%T corresponds to an absorbance of 0.2024. Use the table to verify this.

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TABLE 1.   **PERCENT TRANSMITTANCE-ABSORBANCE CONVERSION TABLE**

T(%)	ABSORBANCE				T(%)	ABSORBANCE			
	1*	2*	3*	4*		1*	2*	3*	4*
	(0.0)	(.25)	(.50)	(.75)		(0.0)	(.25)	(.50)	(.75)
1	2.000	1.903	1.824	1.757	51	.2924	.2903	.2882	.2861
2	1.699	1.648	1.602	1.561	52	.2840	.2819	.2798	.2777
3	1.523	1.488	1.456	1.426	53	.2756	.2736	.2716	.2696
4	1.398	1.372	1.347	1.323	54	.2676	.2656	.2636	.2616
5	1.301	1.280	1.260	1.240	55	.2596	.2577	.2557	.2537
6	1.222	1.204	1.187	1.171	56	.2518	.2499	.2480	.2460
7	1.155	1.140	1.126	1.112	57	.2441	.2422	.2403	.2384
8	1.097	1.083	1.071	1.059	58	.2366	.2347	.2328	.2310
9	1.046	1.034	1.022	1.011	59	.2291	.2273	.2255	.2236
10	1.000	.989	.979	.969	60	.2218	.2200	.2182	.2164
11	.959	.949	.939	.930	61	.2147	.2129	.2111	.2093
12	.921	.912	.903	.894	62	.2076	.2059	.2041	.2024
13	.886	.878	.870	.862	63	.2007	.1990	.1973	.1956
14	.854	.846	.838	.831	64	.1939	.1922	.1905	.1888
15	.824	.817	.810	.803	65	.1871	.1855	.1838	.1821
16	.796	.789	.782	.776	66	.1805	.1788	.1772	.1756
17	.770	.763	.757	.751	67	.1739	.1723	.1707	.1691
18	.745	.739	.733	.727	68	.1675	.1659	.1643	.1627
19	.721	.716	.710	.704	69	.1612	.1596	.1580	.1565
20	.699	.694	.688	.683	70	.1549	.1534	.1518	.1503
21	.678	.673	.668	.663	71	.1487	.1472	.1457	.1442
22	.658	.653	.648	.643	72	.1427	.1412	.1397	.1382
23	.638	.634	.629	.624	73	.1367	.1352	.1337	.1322
24	.620	.6l5	.611	.606	74	.1308	.1293	.1278	.1264
25	.602	.598	.594	.589	75	.1249	.1235	.1221	.1206
26	.585	.581	.577	.573	76	.1192	.1177	.1163	.1149
27	.569	.565	.561	.557	77	.1135	.1121	.1107	.1093
28	.553	.549	.545	.542	78	.1079	.1065	.1051	.1037
29	.538	.534	.530	.527	79	.1024	.1010	.0996	.0982
30	.523	.520	.516	.512	80	.0969	.0955	.0942	.0928
31	.509	.505	.502	.498	81	.0915	.0901	.0888	.0875
32	.495	.491	.488	.485	82	.0862	.0848	.0835	.0822
33	.482	.478	.475	.472	83	.0809	.0796	.0783	.0770
34	.469	.465	.462	.459	84	.0757	.0744	.0731	.0718
35	.456	.453	.450	.447	85	.0706	.0693	.0680	.0667
36	.444	.441	.438	.435	86	.0655	.0642	.0630	.0617
37	.432	.429	.426	.423	87	.0605	.0593	.0580	.0568
38	.420	.417	.414	.412	88	.0555	.0543	.0531	.0518
39	.409	.406	.403	.401	89	.0505	.0494	.0482	.0470
40	.398	.395	.392	.390	90	.0458	.0446	.0434	.0422
41	.387	.385	.382	.380	91	.0410	.0398	.0386	.0374
42	.377	.374	.372	.369	92	.0362	.0351	.0339	.0327
43	.367	.364	.362	.359	93	.0315	.0304	.0292	.0281
44	.357	.354	.352	.349	94	.0269	.0257	.0246	.0235
45	.347	.344	.342	.340	95	.0223	.0212	.0200	.0188
46	.337	.335	.332	.330	96	.0177	.0166	.0155	.0144
47	.328	.325	.323	.321	97	.0132	.0121	.0110	.0099
48	.319	.317	.314	.312	98	.0088	.0077	.0066	.0055
49	.310	.308	.305	.303	99	.0044	.0033	.0022	.0011
50	.301	.299	.297	.295	100	.0000	.0000	.0000	.0000

*Columns 1, 2, 3, and 4 give absorbance values corresponding to the whole % value of T given in the left hand column plus the fraction of a % indicated below the column number (e.g., 1.50 %T corresponds to an absorbance of 1.824).

PIPETTING

Volumetric pipettes

Volumetric pipettes are long glass tubes that are used to accurately dispense a measured volume of liquid. They are made in a variety of sizes and are usually graduated. The total volume and the units of the smallest divisions are usually inscribed on the upper end of the pipette (Figure 4). Two types of pipette are commonly used:

1. *Non-blowout pipette* (Figure 4 a). The scale numbers nearest the tip of the pipette equals the total capacity of the pipette. Dispense the liquid only to this line; do not expel the remaining liquid.

2. *Blowout pipette* (Figure 4 b). The last scale number on this pipette is one unit less than the total capacity. All the liquid in this pipette must be expelled to dispense the last unit.
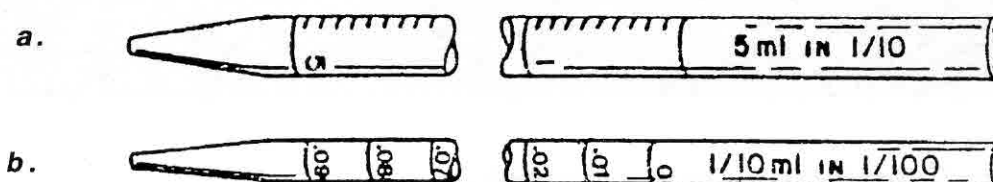
Figure 4. (a) a 5 ml non-blowout pipette with the smallest unit equal to 0.1 ml; (b) a 0.1-ml blowout pipette with smallest unit equal to 0.01 ml. Note: the examples shown here are just to illustrate the two types of pipettes; the types of 0.1 and 5 ml pipettes you use in lab. May differ from these.

Water tends to climb up and wet the walls of glass containers. In narrow tubes, like pipettes, this tendency causes water to form a concave upper surface called a **meniscus**. A pipette is filled when the lower surface of the meniscus coincides with the graduation line of the volume you wish to dispense. Pipetting aids of various types are normally used to safely fill and dispense liquids from pipettes.

To maximize accuracy, always select a pipette with a total volume similar to the volume you wish to pipette. For example, if you wish to dispense 1.0 ml, you use a 1.0-ml pipette filled completely, not a 5.0-ml pipette only one-fifth filled.

Digital Micropipettors

The digital micropipettor is a precision instrument designed to fill a disposable pipette tip with a user-selected volume of solution. Different models of micropipettors allow you to "dial up" in micrometers (μl) the volume you wish to pipette within the volume range that the model provides. You will be using two models of micropipettor in this course: a 2-20 μl micropipette and a 20–200 μl micropipette.

Recall that there are 10^3 μl is a milliliter (ml) and 10^6 μl in a liter (l). Complete the following conversions:

1 μl = _____ ml   100 μl = _____ ml   0.001 l = _____ μl

Principle of Operation

A typical digital micropipettor consists of a barrel that is grasped in one hand, a shaft on to which disposable pipette tips are mounted, a knob for adjusting the volume being transferred, a plunger for filling and emptying the tip, and a tip ejector mechanism. After setting the volume adjuster (do not set this if the

knob is under 'lock' position, first change it by flipping it to 'unlock' mark) to the correct volume and inserting a disposable pipette tip onto the micropipettor, the user depresses the plunger on the top of the barrel to the first, "calibrated stroke" position. The plunger pushes a piston into a cylinder a distance that is determined by the volume setting. When the plunger is released, the piston is pulled out of the cylinder and a defined volume of fluid is drawn into the pipette tip. The plunger is than depressed to the first position and on to a second, "blow out" position that dispenses the fluid out of the tip. If appropriate, the tip can either be reused, or ejected from the micropipettor. Most micropipettors are designed for one hand operation.

Pipetting Instructions

The following instructions are for the Oxford® *BenchMate* micropipettor (Figure 5):

Figure 5. The Oxford® *BenchMate* digital micropipettor.

1. *Set Volume.* First loosen the lock nut mechanism at the base of the plunger by turning it counterclockwise (Figure 5 left). Now turn the thumb screw at the end of the plunger clockwise to decrease the volume setting and counterclockwise to increase the volume setting. Be sure you understand how to read the digital display (Figure 5 right). The white horizontal line on the display represents the decimal point. DO NOT attempt to rotate the volume adjuster beyond the upper or lower limits of the pipette's range, as stated on the top of the barrel. When you have properly set the volume, turn the thumb screw to lock the instrument at that volume.
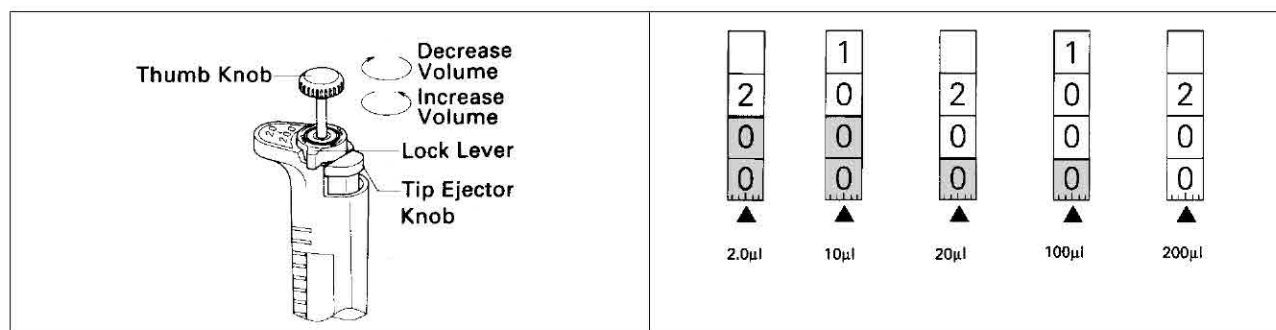
Figure 6. Left: mechanism for setting volume. Right: digital scales for various models. Note: grayed part of scale indicates decimal portion of reading.

2.   *Fill the tip.* Firmly insert a new tip on to the stem. Before inserting the tip into the solution, use your thumb to depress the plunger to the first position (Figure 6). Now, insert the pipette tip into the solution to a depth of not more than 3 mm. <u>Smoothly</u> return the plunger to the release position, drawing liquid into the tip. Withdraw the tip from the container.
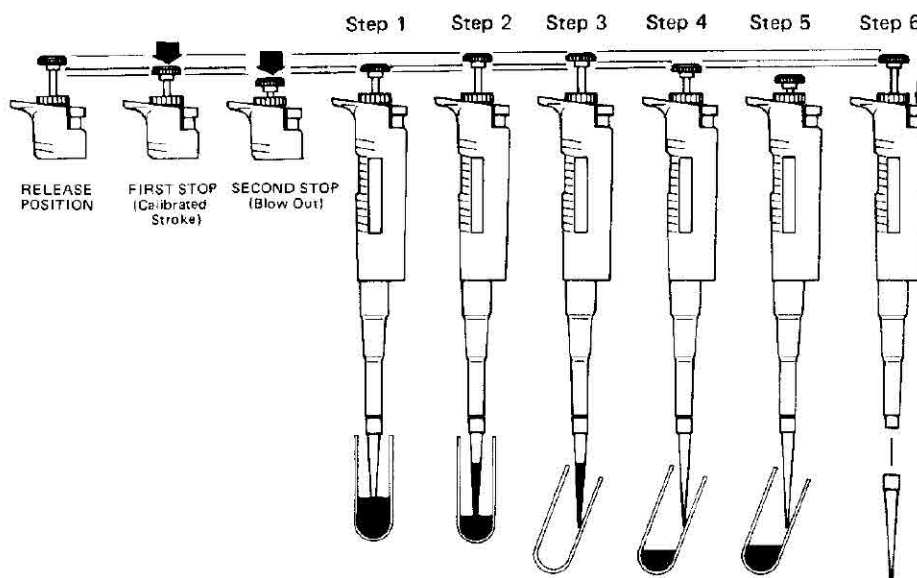
Figure 7. Filling and expelling a sample from the digital micropipettor. See the text for a detailed description of the procedure to use.

3.   *Expell the Sample.* Place the pipette tip against the side wall of the container to receive the sample. <u>Smoothly</u> depress the plunger to the first stop. Pause briefly, and continue to depress the plunger to the second position. With the plunger still depress, slide the tip out of the container, leaving the sample on the side wall near the bottom.

4.   *Eject the tip.* Gently, return the knob to the release position. Do not allow the plunger to "snap" back. Remove the disposable tip by firmly depressing the tip ejector button.

The following techniques aid in pipetting accuracy and reproducibility.

a.   Smooth, consistent depression and release of the plunger insures an even intake and release of liquid from sample to sample. Never allow the plunger to "snap" back.

b.   To avoid bubble formation in the tip, always depress the plunger to the "calibrated fill" position before inserting the tip into the sample.

c.   Insert the tip to about the same depth in all samples (about 3 mm). Hold the instrument as vertically as possible as you fill the tip. [Tip: It helps to use one hand to support the other hand that is holding the instrument.]

Precautions

The digital micropipettor is an expensive, precision instrument. Please follow these precautions in use of this piece of equipment.

1.   Never attempt to rotate the adjuster know while it is in lock position.

2.   Never attempt to rotate the adjuster knob beyond the upper or lower limits for the pipettor's range.

3.   Never attempt to fill the micropipettor without a tip in place.

4.   Never allow the plunger to snap back when withdrawing or ejecting a sample.

5.   Never lay down the pipettor with a filled tip.

6.   Never immerse the barrel of the pipettor into liquid.

Pipetting Exercises

In these exercises you transfer three aliquots of different volumes into a container. You then set the micropipettor to withdraw a sample that is equal to the total volume of liquid that you added to the container. If your pipetting is accurate, the sample should just fill the pipette tip without a bubble and with no sample left over. Each exercise uses a different pipetting model. You will be pipetting distilled water to which food coloring has been added.

Small Volume—use the 2–20 μl model.

Adjust the micropipettor to add 2 μl, 5 μl, and 10 μl to a tube. You will need to "pool" the sample by briefly spinning the tube in a balanced micro-centrifuge. Set the micropipettor to 17 μl and withdraw the sample from the tube.

Large Volume—use the 20–200 μl model.

Adjust the micropipettor to add 20 μl, 50 μl, and 100 μl to a tube. Set the micropipettor to 170 μl and withdraw the sample from the tube.

APPENDIX 3 – SCIENTIFIC WRITING AND POSTER PREPARATION

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Revised June 2010 – Mark A. Sarvary

INTRODUCTION

When a researcher embarks on a scientific investigation, it is often expected that the final outcome will be written up and submitted for publication at a peer-reviewed journal. What is peer review? Once a paper is submitted to a scientific journal, it is passed out to other scientists in that field. A scientist who is given a paper to review for a journal might comment on anything from grammar, to techniques used, to references cited, to the way in which data were analyzed. Peer review is the mechanism by which the quality and credibility of scientific research is maintained and respectable scientific journals require all papers to pass peer review before they are accepted for publication. Granting agencies also choose which proposals to fund based on the opinions of peer reviewers who work in committees to select the top research projects. You will gain experience with process of peer review in looking at your fellow students' work in later assignments.

Most journals require that results be written up in a format that includes at least 5 sections: Introduction, Methods, Results, Discussion and References. This format makes it possible for other scientists to verify the results and build on new knowledge gained. You can usually recognize a primary article reference, the original scientific report of new research findings, by looking for the 5 sections listed above. You will also have use this format in your poster presentations. We'll look at the scientific communication process in more depth later in this chapter and in the references at the end and you'll get more information and practice in the tutorials at http://mannlib.cornell.edu/instruction/tutorials/.

However, before an article is finalized, researchers often take opportunities to attend professional conferences to exchange ideas with other participants. Traditionally one would use 15-30 minutes to orally present his or her data and receive feedback from the audience. Because of many advantages both for presenters and the conference organizers the trends of presentation in recent years have been in the form of posters. Although the length of the writing in a poster is much shorter than that in an article prepared for publication, the writing style between these two forms of information delivery is often quite similar. One fundamental difference is the requirement of extra visual considerations in poster design. In this chapter we will consider many aspects one should consider in preparation for a scientific poster. The first part is devoted to the writing portion of the poster; the later part covers visual aspects of the design. Because the poster has been the leading trend for presentation, there are reports, books and individual articles posted on the web to guide those who are in need of help. There is also a list of such resources at the end of this chapter that may provide ideas in your preparation.

The following suggestions regarding writing and designing a poster require access to certain computer software, for example, Microsoft Excel® and PowerPoint®, and some knowledge of using them. Documents describing basic operational procedures for these two programs can be downloaded from BioG 1101-1104 website at http://biog-1101-1104.bio.cornell.edu/BioG1101_1104/default.html. Please note that the University has moved to Microsoft Office 2007, which is considerably different from Office 2003. Although familiarity with this software is essential in creating a successful poster, learning to use it should not be a difficult task. If you do not have access to either a personal computer or the software, Mann Library, located at the Agriculture Quad off Tower Road on campus, is equipped with both the hardware and the software for creating posters. You can also get additional training in using these programs from Mann at http://mannlib.cornell.edu/instruction/workshops/ and from CIT (see the Cornell Information Technologies Training page at http://www.cit.cornell.edu/training/). Mann also provides

services for printing out the large-format posters, which is the final group assignment to be turned in toward the end of the semester.

PLANNING YOUR POSTER

Writing and designing a good poster can take many weeks of preparation! It is important that you start early and work collaboratively with your teammates. Divide the labor within your group and assign each one the tasks of writing particular sections of the poster. Bear in mind that after the full text and graphics are complete they need to flow well and appear consistent from one section to another.

Data Gathering

When conducting the osmosis research with your group you should record data thoroughly and start writing the methods and results sections as early as possible. Details in these two areas can be easily forgotten, making the analysis difficult. Once you have completely filled in the data they can be used as a great starting point for writing the text of the poster. Use the Data Collection Form to write your method section. The Raw Data Table can help you start the statistical analysis. The information filled in the Study Proposal Form will be useful for the introduction portion of the writing.

Writing

Among the most important requirements in scientific writing is its clarity and concision. A complex sentence structure is usually not encouraged. You should write to an audience who has a similar biology background as you but was not there at the lab with you. The purpose is to make him/her understand what your question was, what you did, and what your conclusion was without having been there. So the writing will start with a broad description and becomes more focused as the audience reads on. The poster will conclude with a good discussion and interpretation of the research outcome. Your reader should be, for the most part, able to replicate your study after reading your writing. You may already have experience writing lab reports, articles or poster presentations that include the 5 sections required for proper scientific writing (Introduction, Methods, Results, Discussion and References); however, even senior scientists continue to work at improving their writing and the key to clear writing is good editing. It is essential to become a good editor of your own work, but sometimes someone else can identify problems with a paper that the writer did not see. For this reason, senior scientists show papers to colleagues before submitting them to a journal. In order to improve your writing and editing skills, you will also be required to peer review your classmates papers for a few writing assignments. You will also find useful references at the end of the chapter to additional scientific writing resources.

Poster Design

To get an idea about what a scientific poster looks like a few examples will be available in lab and your lab instructor will explain what is expected of you in the poster making process. In the meantime, you can also check the references at the end of this chapter for examples and templates, particularly the online tutorials from Swarthmore and North Carolina State University. You can also visit a research department building on campus to see many examples posted on the walls outside research laboratories. Find a morning or afternoon and spend 15 to 30 minutes for a survey. To name a couple, the Corson-Mudd Halls are home to Neurobiology and Behavior Department and Ecology and Evolutionary Biology Department. On the walls of various hallways and different floors are research work presented by students and faculty. Take a look around the various designs and write down some notes about why you prefer one to the others. One of the differences you will experience when you start to work on your own is that you may not have as many figures and tables as most of the posters you see there. The reason is

quite simple. Most of the research posters you see there are loaded with data gathered from months and years of work. You are only going to use up to three hours to gather yours. So do not get intimidated by their data-rich presentation. Your poster will still be very valuable as an experience to learn how to do science.

Once you have seen a few examples, sketch an outline of what yours is going to be. You will have a few graphics, but not too many. The main coverage of your poster will be the text. How do you make it appealing? You may want to show a picture of your study. If so, bring a camera to the lab and take a few photos. Photos from websites tend not to reproduce well because the images are not of high enough quality. Besides, there is copyright issue that you need to consider, so you should create your own images whenever feasible.

<u>Large-Format Printing</u>

The large-format poster is not due until toward the end of the semester. Processing each poster for printing may take a minimum of half an hour and as long as several hours of waiting on campus facilities. <u>The paper quality of the poster will not affect your grade, therefore use the most affordable!</u> If you use off-campus businesses it may take overnight without the staff to assist you with final details. In addition, the last printing of the day starts about an hour before closing. With all these factors compounded, you should realize that printing out your final work is not a last minute task. You and your group may easily miss the deadline if there is not enough planning. Please be very mindful of the schedule. Budget at least two-three days in advance for the visit before your due date.

There are a few print outlets with large-format plotters on and off campus. In general services provided on campus are excellent. Campus facilities can often provide staff to work with you to make sure that your print job is successful so that the result is as close as possible to what you have expected (the difference between colors and images on screen versus in print can be quite marked). Check their <u>summer hours</u> as part of your poster-making plan. Note: it is not necessary to use the more expensive glossy paper. The regular bond paper will be sufficient.

Mann Library Computing Services: First floor. Tel: 255-3240
Of the 20 libraries on campus, Mann Library is the one that particularly serves the students, faculty, and staff of the College of Agriculture and Life Sciences, the College of Human Ecology, and the Division of Nutritional Sciences. Mann provides resources and assistance which will help you be successful not only as you complete your work for this class but also throughout your career at Cornell. The library has not only hardware and software to help complete your assignments, including computers (desktop and laptop), programs like PowerPoint and Excel, and large-format plotters for poster printing (http://www.mannlib.cornell.edu/computing/) but also:

•   Millions of resources (books, articles, and more) both in print and available online through the Library Gateway (http://library.cornell.edu/)
•   Personal assistance from the reference staff to help answer any questions you have (http://www.mannlib.cornell.edu/services/reference/index.cfm)
•   Workshops to learn how to research efficiently and to use PowerPoint and other programs to present your research (http://www.mannlib.cornell.edu/instruction/workshops/index.cfm)

The experienced staff in the Stone Computing area will work with you at one of their computer workstations to make sure that your poster prints correctly (though they do not have expertise in poster design). Look at the instructions on printing to the color plotters, either online at http://mannlib.cornell.edu/computing/printingandscanning/printing/plotter.cfm and be sure to check

with the computing center student operators at the desk on the first floor of Mann Library before you print. There are also some online resources on poster design and printing listed at http://www.mannlib.cornell.edu/instruction/tutorials/index.cfm. The regular hours are: 8 AM to 8 PM from Mondays through Thursdays, 8 AM to 5 PM on Fridays, noon to 6 PM on Saturdays and closed on Sundays. Current hours, particularly for breaks and holidays, can be found at http://www.mannlib.cornell.edu/hoursandmaps/. Please note: Mann does not allow any plotter print jobs to start after one half hour prior to closing; during unusually heavy use times when there are many poster "jobs" lined up on the network, access to the plotters may be restricted an hour or more before Mann Library closes.

Website: **http://www.mannlib.cornell.edu**
Reference Services: **(607) 255-5406, mann_ref@cornell.edu, Ask a Librarian**
                                          online chat (http://library.cornell.edu/services/askalib.html)

Architecture Department: 201a Rand Hall. Tel: 255-4620
Designed to assist students in Architecture Department, the print facilities are open to other users on campus as well. Their costs for printing are comparable to those of Mann Library. They are staffed Mondays through Fridays 8:30 AM to 5 PM. Call for summer hours before your visit.

FedEx Kinko: 605 W. State Street. Tel: 272-0202
FedEx Kinko is located downtown Ithaca at the corner of Meadow Street. They open 24 hours during weekdays (except for Friday nights) and 9 AM to 9 PM Saturdays and Sundays.

Gnomon Copy: 732 S. Meadow Street. Tel: 273-3333
This copy center is located in a shopping complex with other large retail stores including Pier 1 and Michaels. Their hours are 8 AM to midnight Mondays through Fridays, 10 AM to 6 PM on Saturdays and 1 PM to 9 PM on Sundays.

TEXT FORMAT OF THE POSTER

Although the format of a poster enjoys quite a bit of flexibility compared to a paper prepared for publication, it is still commonly divided into the same sections found in a scientific paper, with each section containing much shorter, concise information about the study. It often starts out with an informative title, a line about the author(s), and their affiliation(s). The main body of the text is divided into Introduction, Methods, Results, Discussion, and References plus the Abstract. Just as a paper in which the Abstract is a stand-alone section, the Abstract of a poster in recent years is often submitted to the conference separately and is not included in the poster design. Therefore, for your assignment, the abstract should be written individually and not included in the poster. The total word count, not including the abstract, should be about 800 to 1000 words.

In addition to the suggestions below, a few samples will be used for discussion in class.

TITLE

The title of a poster is a short description of the study and is what most viewers see before they read the other parts of the poster. It should be clear, concise and serves as the ultimate summary of the study. Many people use a catchy or humorous approach to phrase the title. It is a good strategy because a well-phrased title can draw readers to read on what the study is. However, bear in mind not to let the title say more than what the study actually is.

INTRODUCTION

The Introduction section describes the background of the research and presents the objective of the study. It puts forth the explanatory system and justifies the reason in choosing the specific organism as the research subject. For a research project with many aims, each one of them can be presented as a separate bullet point, in bold print, or in a separate color. Otherwise, for a relatively straightforward study like ours the purpose should be stated clearly at the end of the paragraph. The scientific name(s) of the subject should be included in this section. Any definition that is not common knowledge should be defined in this section. Well-established facts are written using the present tense.

METHODS

The Methods section concisely describes what was done, so the writing should be in the past tense. It should not be extensive but should point out the basic experimental design. The number of the experimental organisms, the number of replications, and the statistical analysis are all important components of a study and should be included in this section. In general, established techniques and common equipment should be mentioned without detailed description. Once described, their acronyms should be used instead of their full names. However one should not mention extremely common equipment such as a compound microscope except under special circumstances. If the designs and procedures were modified from another publication, include the citation in the paragraph. If the space permits, a picture of the experimental device can be included.

RESULTS

In a poster the results section is often more visual than other sections because of data presentation using figures and charts. Nevertheless the writing in Results can complement what the graphics cannot deliver. The writing in this section should include the observed data and the analyzed outcome. There should not be interpretations of the analysis. Spatially, the graphic elements related to the Results section should be positioned so that one can see the connection between them. Just as in the Methods section, the sentences in Results should be written in the past tense. If statistical tests were used, their significance should be mentioned here.

DISCUSSION

This is where all the interpretations should be. Use this space to explain why the results were the way they were. Were the results consistent with the research hypothesis? If so, what does it mean? If the results were not significant statistically, what are the possible explanations?

ABSTRACT

Usually written at the last stage of the process, an abstract is the summary of the entire study. It highlights all the important information of the study, including the scientific names of the study organisms, rationale of the study, a brief mention of methods, a short description of the results, and a concise conclusion. No reference to figures or tables should be included in the abstract. It is also uncommon to cite other studies in the abstract. The abstract is an <u>independent</u> section and should provide a sufficient amount of information to give a general picture of the study to the readers even if they are not going to read the entire poster. An abstract prepared for a conference is often submitted to the organizer in advance. The collected abstracts along with conference agenda and other information are distributed to the participants at the first day of the conference. Because of this, most posters these days do not include an abstract as it is redundant to do so.

Although conducting the osmosis study and designing a poster is a group project, each member of your group should write his/her own abstract <u>independently</u>. It is prepared as if it will be submitted for the conference catalog, so it will not be part of the poster. The abstract should be no more than half a page long, typed (not hand-written), double-spaced, with a 12-point size, and one inch margin.

FIGURES AND TABLES

Figures and tables are a very important part of a poster. Well prepared tables or figures save a lot of writing, which in turn save the limited space available in posters. They also should be stand-alone and can express a lot of information without readers resorting to the text. Figures and tables are numerically labeled respectively as Figure 1, Figure 2, or Table 1, Table 2 and so on. The title and short description of each, called table legend or figure legend, is placed differently in the two types of data presentations. The table legend should be put <u>above</u> the table while the figure legend should be placed <u>below</u> the figure. The first portion of the legend is the **title.** If a title provides sufficient amount of information the legend is complete without further description. If further description is needed, they should be written in complete sentences. As the standard in scientific data presentation, the measurement units should follow the metric system. If there are abbreviations in the table or figure, their full definition should be included in the legends.

In a figure each axis should be labeled with concise information pertaining to that variable including unit if appropriate. The following is an example on call behavior in field crickets *Gryllus assimilis* based on fictitious data. Notice that in the figure legend the title is followed by a description of each of the graphic symbols.

In addition to the main sections of the text, scientific writing, either a paper or a poster, has a few additional sections.

Table 1. Average day and night transpiration rates (mg H_2O/cm^2-hr) and ratios of the day and night rates of water-sufficient and water-stressed plants (Adapted from Allen 1980).

Week of Study	Treatment Group					
	Water-Sufficient			Water-Stressed		
	Day	Night	Day/night	Day	Night	Day/Night
1	0.43	0.90	0.48	1.44	1.17	1.23
3	1.40	1.21	1.16	0.50	0.84	0.68
5	2.10	2.10	1.00	0.56	1.63	0.88
7	0.67	0.28	2.39	0.08	0.12	0.67
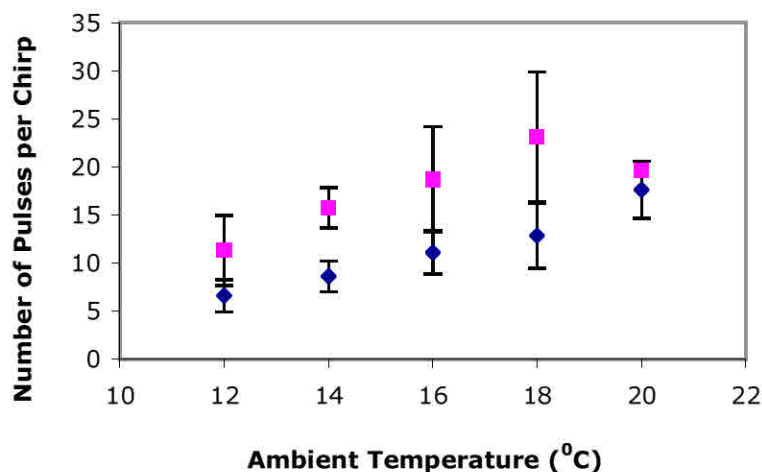
Figure 1. The relationship between the number of pulses per chirp and the ambient temperature in field cricket *Gryllus assimilis* found on the east and west sides of Cayuga Lake. The squares represent mean number of pulses in the population east of the lake and the diamonds are mean values of the western population. The vertical bars are ±1 standard deviation.

REFERENCES

Since knowledge of the explanatory system in a specific area of science is used to interpret new observations and formulate hypotheses, remaining well-informed and knowledgeable about and citing the published literature are important activities for biologists. Accessing and managing the information contained in research libraries are skills that all scientists must develop. In keeping with the objective we have to give you experience with all aspects of modern biological research, we have included several activities and assignments this year that are designed to acquaint you with Mann Library and online access to its holdings. Mann Library, being the major repository of the biological holdings in the Cornell University library system, is a resource you will be using frequently during your undergraduate years.

Library research at the university level is a whole new world. For success in your assignments and in your future college career, a web search or two won't do for finding comprehensive and authoritative scholarly information in the biological sciences. Mann has created tutorial assignments to help you learn how to search the library resources available at Cornell fast and efficiently for the articles you will need. The tutorials are available at http://www.mannlib.cornell.edu/instruction/tutorials/.

All scientific work was built on knowledge previously gathered by others. That is why it is important to list those publications that have immediate relevance to yours. If others do not know or cannot find the sources you have used, a link in the chain of scholarly conversation is broken. In addition, properly citing the sources of work you have used gives credit to the authors and avoids plagiarism (whether unintentional or intentional), which is part of the Cornell Code of Academic Integrity. Use the format presented at the end of this or any other chapter in *Investigative Biology* and list any published work from which information for your study was drawn; this citation format is the American Psychological Association (APA) style. You can find a short guide to this style online at **http://www.library.cornell.edu/t/help/res_strategy/citing/apa.html** and most references desks will have a print copy on their reference shelf. It is important to use the same citation style consistently. All listed citations should be referred to at least once in the main text where appropriate. At the same time any literature mentioned in the text should be included in the References section. Inclusion of published work is common in Introduction, Methods, Results, and Discussion, but usually not in Abstract. The name of this section varies among journals. In addition to References, some journals may use Literature Cited as an alternative while other, Citations, or Cited References. For our purposes you may use References as a section for the published work closely relevant to your study.

To include citations in the text, place the citation immediately following the statement of the citation.

> from theory on bounded rationality (Simon, 1945)
> Simon (1945) posited that
> as has been shown (Leiter & Maslach, 1998)
> Kahneman, Knetsch, & Thaler (1991) found [for the first reference to multiple authors; thereafter use Kahneman et. al. (Latin for "and others") and publication year]

To cite a specific part of a source (always necessary for quotations), include the page, chapter, etc. (with appropriate abbreviations) in the in-text citation.

> (Stigter & Das, 1981, p. 96)

> De Waal (1996) overstated the case when he asserted that "we seem to be reaching ... from the hands of philosophers" (p. 218).

If page numbers are not included in electronic sources, provide the paragraph number preceded by the paragraph symbol or the heading and following paragraph.

> (Mönnich & Spiering, 2008 ¶ 9)

If more than one reference is used in a statement, follow the order in which they appear in the reference list (alphabetical by last name of first author usually), then chronological order if citing multiple works by the same author.

> Several studies (Edeline, 1991; Johnson, 1991, 1993; Markson & Busch, 2000)

To prepare a References section, first follow the alphabetical order of the last names of the first authors. If the same author(s) published multiple papers or books, list the publications in a chronological order. All authors are listed by their last names, followed by their initials. The published year immediately follows the listing of authors. Aside from these general rules, the format of each citation varies depending on whether the source is an entire book, a book chapter, or a journal article.

- Books: References to an entire book must include the following elements: author(s) or editor(s), date of publication, title, place of publication, and the name of the publisher.

> Dawkins, R. (1976). *The selfish gene*. Oxford, England: Oxford University Press.

If newer edition, add the number of edition after the book title.

> Dawkins, R. (2006). *The selfish gene*. (2nd ed.). Oxford, England: Oxford University Press.

- Chapter of a book with different author(s): References to an essay or chapter in an edited book must include the following elements: essay or chapter authors, date of publication, essay or chapter title, book editor(s), book title, essay or chapter page numbers, place of publication, and the name of the publisher.

> Tauber, C.A., & Tauber, M.J. (1989). Sympatric speciation in insects: Perception and perspective. In: D. Otte & J. Endler (Eds.) *Speciation and its consequences*. (pp. 307-344). Sunderland, MA: Sinauer.

- Journal articles:

> Hyatt, K.D., McQueen, D.J., Shortreed, K.S., & Rankin, D.P. (2004). Sockeye salmon (*Oncorhynchus nerka*) nursery lake fertilization: Review and summary of results. *Environmental Reviews*, 12:133-162.

> Suydam, I.T., Snow, C.D., Pande, V.S., & Boxer, S.G. (2006). Electric fields at the active side of an enzyme: Direct comparison of experiment with theory. *Science*, 313:200-204.

- Internet articles based on a print source (exists in print and online)

> Ku, G. (2008). Learning to de-escalate: The effects of regret in escalation of commitment [Electronic version]. *Organizational Behavior and Human Decision Processes*, 105(2), 221-232.

- Article in an Internet-only journal

> Moerman, D. E. (2003, June). "Placebo" versus "meaning": The case for a change in our use of language. *Prevention & Treatment*, 6(1). Retrieved May 6, 2008, from
> http://psycnet.apa.org/index.cfm?fa=search.displayRecord&uid=2003-07872-007

-Journal article from a database

> Choi, J. (2008). Event justice perceptions and employees' reactions: Perceptions of social entity justice as a moderator. *Journal of Applied Psychology*, 93, 513-528. Retrieved May 6, 2008, from PsycARTICLES database.

- Internet: As the Internet has become a popular source of information, proper citation format should be followed. The following is an example of how to list a source from the Internet. However, for your study you should stick to publications from peer-reviewed journals.

> Centers for Disease Control and Prevention (US). (2007). *Key Facts About Avian Influenza (Bird Flu) and Avian Influenza A (H5N1) Virus.* Retrieved June 15, 2007, from http://www.cdc.gov/flu/avian/gen-info/facts.htm

If the type of publication you wish to cite is not listed here or in the references in *Investigative Biology*, please see the full guide: American Psychological Association. (2001). *Publication manual of the American Psychological Association* (5th ed.). Washington, DC: Author. The 5th edition is available from a number of libraries on campus, including Mann Library; do a title search in the Library Catalog. Many of these are at reference desks which means they are not available to be checked out; however, they can be consulted at the desk and you can ask a librarian for help. There are also a number of short guides from libraries available online, including on the Library Gateway at http://www.library.cornell.edu/t/help/res_strategy/citing/apa.html.

For more information on how to cite properly, see the tutorial on recognizing and avoiding plagiarism at http://plagiarism.arts.cornell.edu/tutorial/index.cfm. For tools to make gathering and citing your resources easier like RefWorks (online) and EndNote (software available for purchase at the Campus Store), see the library's listing of citation tools at http://campusgw.library.cornell.edu/services/citing.html. These programs will be especially helpful for easy management and citation of your references when you begin to do longer projects and papers.

ACKNOWLEDGMENT

In a scientific study often there are people or institutions involved that have made the study possible. This is where you should express your appreciation. If the study was funded by others, list the name of the agency and the grant number. Because of the nature of the lab, it may not be necessary to include this section in your poster.

APPENDIX

An appendix may be used in some journals for necessary information that does not fit into the accepted format for a paper. Normally, due to limited space, the appendix is not included in a poster. To ensure accuracy of your statistical tests, an appendix is usually prepared separately from the poster and on separate sheet(s), and include the following information:

- The name of the test and a precise description of the data compared in the test
- A table showing how the data were treated to calculate the **test statistic**
- A statement of H_o and H_a
- The tabular statistic with its alpha level (and degrees of freedom, if appropriate)
- Comparison of test and tabular statistics, and conclusion of the test

In this study your Osmosis/Statistical Testing worksheet functions as your appendix, therefore you do not need to turn in an additional appendix**.**

VISUAL ASPECTS OF THE POSTER

There are many other considerations when you develop a poster in addition to the writing style described earlier. Because a poster is a visual representation of your study, it is important to create an informative and aesthetically appealing poster so that the readers can quickly grasp what is being studied. As the format of posters varies from conference to conference, we will simplify the preparation by providing a standard that is generally followed by many conferences and suitable for the course.

PRESENTATION OF THE TEXT

It is often thought that a poster should be made so that its main text and graphics can be easily read from a distance of 2 to 5 feet and its title can be read from 10 feet away. With this in mind the choice of typeface and size is critical. Here is a list of suggested typeface and size and related choices.

1. Typeface. It is recommended that a non-serifed typeface is a better choice for the poster title and subheadings. For the main text a serifed font may be easier to read. A serifed font, marked by the crosslines at the ends of a stroke in each character, can guide the eyes and make the reading easier in regular text size. In a poster it is not recommended to have more than two typefaces so that readers are not distracted by the differences between them. One should avoid using novelty types as they often distract readers from the main purpose of the study.

   Examples of serifed type: Times New Roman, Book Antiqua, Palatino
   Examples of non-serifed type: Helvetica, Arial, Comic Sans

   Each typeface also comes with choices of regular, bold, italicized and underlined varieties. Appropriate use of different type variation can often highlight important points in a poster and make the poster easier to read. The italicized variation of a font usually works better than the underline type in a poster.

2. Type size. Size of types varies based on the size of the poster and whether it is used in the title, subheading or the main text. For the purpose of the lab assignment we recommend a poster with dimensions about 42" x 36". The following suggested type sizes are available from Microsoft PowerPoint® and are appropriate for the recommended poster size:

   Title: 62–72
   Author line: 44-54
   Affiliation: 44-54

Subheading: 44-54
Main text: 28
References: 24

3. Line length and spacing. There is a limit on how long a line of text should be before tiring out the readers. The ideal length of text line should be between 20 to 60 characters. The lines should be single-spaced.

4. Number of text lines. During preparation, one should bear in mind that a poster is not a printout of a manuscript pasted on a poster board. One of the major differences when laying out text in a poster is the limit of how many lines the reader can read comfortably. A block of text should not exceed 10-15 lines. Too many lines in a block of text often appear monotonous and can easily cause fatigue. Using graphics is often helpful in breaking the monotony and creates a better aesthetic effect.

GENERAL LAYOUT

Just like many other visual considerations, the layout of a poster can be a matter of taste. But there are some general expectations. The title should be set high on the top of the poster. All capital letters in the title may draw attention and yet make it hard to read at the same time. A mix of capital and lower case may be a better choice. As a western language, English is often laid out following a direction of left to right and up and then down. Consequently, to begin reading the poster one often expects to see the introduction section located at the upper left corner. The results and conclusions are often found at the right column. This design is especially common in the poster in which the orientation is more horizontal (landscape). An example is shown in Figure 2A. If you choose to use the vertically oriented layout (portrait), sections of the text and graphics may be set somewhat differently (Figure 2B).
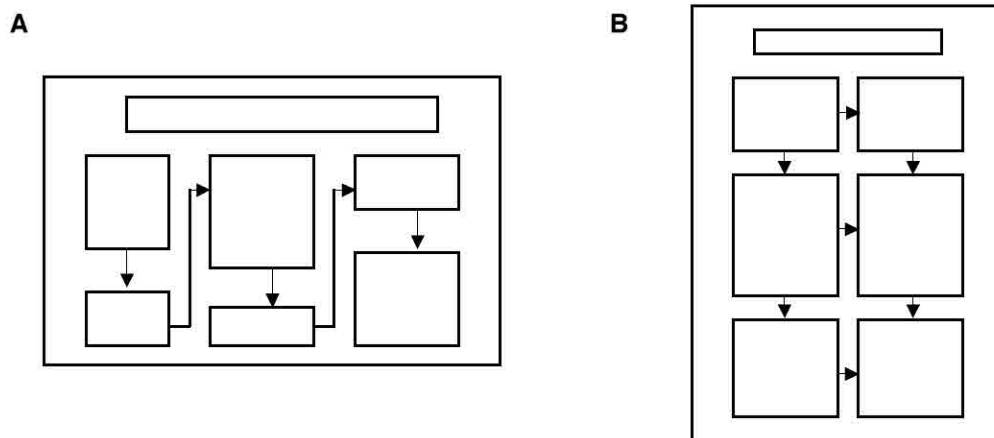
Figure 2. Examples of section layout in a poster of A, horizontal, or B, vertical orientation. The arrows indicate a likely expectation from readers for the flow of sections.

Arrangement of sections in a poster is not limited to the examples above. The examples in Figure 3 may give the readers a more dynamic impression not found in the previous examples.
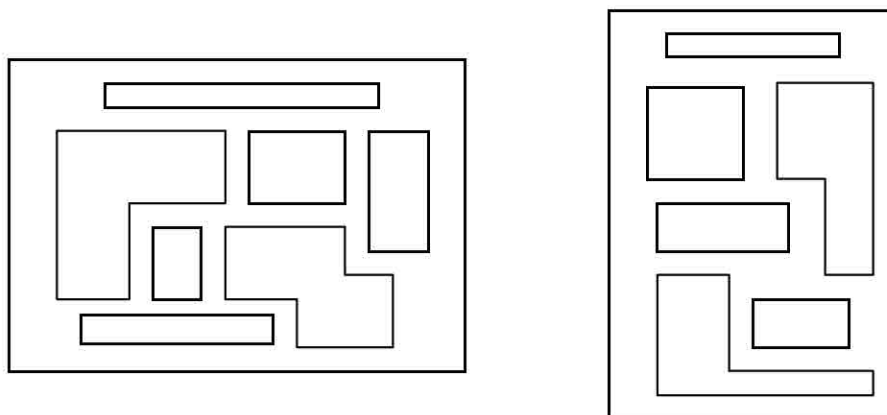
Figure 3. Examples of poster section layout in which some sections are in irregular shapes.

USE OF COLOR

Skillful use of color can turn a monotonous display into a vibrant, delightful presentation. A pure black-and-white poster is rarely appealing. But a colorful arrangement may not necessarily deliver the information well. The bottom line is to choose wisely the colors to properly complement and contrast different sections of the textual and graphic components. In the end, the choice of colors should keep a balance between the eye-catching and harmonious so that there is a sense of integrity.

There is something about colors and emotions:

1. Warm colors such as red, orange and yellow are aggressive and can catch attention and are less settling.
2. Cool colors such as blue and violet are soothing and calming.
3. Whether it is warm or cool, if a color has a high chroma, meaning containing less white component, it is more vivid. If it is diluted with white it becomes more harmonious, less aggressive and also less noticeable. If diluted with black then the color will evoke a sense of stability.

A good choice for the poster background will be a neutral and subdued color. Many people use either off white or dark earth-toned colors. Both should work well as these are often expected colors by the audience. Sections of the text can be highlighted using a color very different from the background. On the other hand, sections can also be highlighted with the same colors so that they give an impression of being related. One should avoid juxtaposing contrasting colors with high chroma unless the purpose is to draw attention to the areas. Even with this intention one should practice caution so that the readers are not sidetracked by this arrangement.

Graphics elements can often be highlighted with colors to make them stand out from the background. Often this can be achieved through simply adding a frame. Colors can also be used in headings and make them different from the main text. This can break the monotony and mark the sections clearly.

REFERENCES AND SUGGESTED READINGS

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LabWrite Project. (2000). *The parts of a laboratory report*. Retrieved July 1, 2008, from http://www.ncsu.edu/labwrite/res/res-studntintro-labparts.html

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Purrington, C. (2008). *Advice on designing scientific posters.* Retrieved July 1, 2008, from http://www.swarthmore.edu/NatSci/cpurrin1/posteradvice.htm

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*Behavior/Ecology is a journal of selected articles based on investigations conducted by BioG 104 students. Three volumes were published in 1978, 1979, and 1980.

Examples of Abstracts

Abstract 1 summarizes a behavioral-ecological field study on a local frog species. Abstract 2 summarizes an enzymological study involving the effect of pH of the enzyme-catalyzed reaction you study in the ENZYMES laboratory. Comments and a brief analysis follow each abstract.

<u>Abstract 1</u>   (from Rosenblum N. 1979. The effect of air-, water-, and mud temperature on the peep rate of the northern spring peeper (*Hyla crucifer*). Behavior/Ecology 2:31-36.)

This study was conducted in order to determine the relationship between air-, water-, and mud temperature and the peep rate of the northern spring peeper (*Hyla crucifer*). Data were collected between 10 p.m. and midnight (EST) on nine different precipitation-free nights during March, April, and May 1978 at the same location in a marsh in the Cornell Research Park near Tompkins County Airport, Ithaca, New York. We measured air-, water-, and mud temperature using mercury bulb thermometers, and with the aid of a stopwatch counted the number of peeps per frog in one-minute periods. The Spearman rank correlation test showed that the number of peeps per frog per minute was significantly positively correlated with all three variable temperatures. Peep rate and mud temperature were the most highly correlated (r_s = .892), while calling rate showed lesser correlation to air temperature (r_s = .691) and water temperature (r_s = .746).

Abstract <u>1</u>: <u>Comments</u> <u>and</u> <u>Analysis</u>

1. The first sentence states the purpose of the study. It could be more concisely written: The relationship between . . . spring peeper (*Hyla crucifer*) was studied.

2. Sentences 2 and 3 summarize the general procedures. Note how concisely the procedures are described. Since this was a field study it is necessary to mention the location of the study site (especially in the <u>Methods</u> section of the paper). It is not necessary to indicate the location of laboratory studies.

3. Sentences 4 and 5 concisely and precisely state the results of the study, including the results of statistical analysis of the data. General conclusions are implied in the statement of results; consequently, they are not stated separately. r_S values are test statistics for the different comparisons (see Spearman rank correlation test in STATISTICAL REFERENCE appendix).

Abstract <u>2</u>

The effect of hydrogen ion concentration on the rate of hydrolysis of p-nitrophenyl phosphate catalyzed by the enzyme alkaline phosphatase from chick intestinal mucosa was determined. Reaction mixtures with pH's ranging from 6.0 to 10.5 were incubated at 37° C for 15 minutes. All reactions were stopped by the addition of NaOH, and the concentration of product formed was determined spectro-photometrically. The rate of the reaction increased with an increase in pH through the pH range of 6.0 to 10.0. A statistical analysis of the results, using the rank sum test, indicated that the difference between the reaction rate at pH 10.0 and the rate at any pH from 6.0 to 9.0 was highly significant (a = .01); however, there was no significant difference between the rates at pH 10 and 10.5. It is concluded that, within the range of pH's used, this phosphatase functions optimally with p-nitrophenyl phosphate as substrate at a pH of 10 to 10.5.

Abstract <u>2</u>: <u>Comments</u> <u>and</u> <u>Analysis</u>

1. The first sentence concisely states the purpose of the study, the reaction studied, the enzyme (and its source) and the substrate used.

2. Sentences 2 and 3 cover the general procedure, but specific details of procedures are not included.

3. Sentences 4 and 5 summarize the results. Also note that sentence 5 states specifically what was being tested in the rank sum test. It was the difference between the rates that occurred at different pH's that was tested for significance. (Do not say: "the difference between pH's was tested.")

4. The last sentence states the major conclusion from the study and restricts it to the scope of the study.

Example of a Scientific Paper

The paper is from a journal of the American Fisheries Society. Comments, explanation, and analysis are presented to the right of the text and below Figure 1. Certain parts of this paper that do not show the form and style required in your writing are indicated and explained.

In general, this paper is well written and organized. It is a good example of the precise and concise writing style required in scientific papers. Notice, however, that even in a paper accepted for journal publication, suggestions for the improvement of the paper can be made. Use the constructive criticisms that your instructor makes on your papers to help you improve your scientific writing skills.

Sun-Compass Orientation in Juvenile Largemouth Bass, *Micropterus salmoides*[1]

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The title indicates the topic under investigation and the research organism used. Note that the scientific binomial is included in the title.

ABSTRACT

Juvenile largemouth bass were tested for manifestations of sun-compass orientation. Fish captured from opposite sides of a pond were tested in a circular pool on clear days and on overcast days. When released from the center of the pool on clear days, the fish swam to the wall of the pool in the compass direction that would have taken them to deep water in the pond from which they had been captured the morning of the test. On overcast days the behavior of the fish from one side of the pond was not significantly different from those from the opposite side of the pond.

The first sentence clearly states what was being tested, although the scientific binomial of the largemouth bass should have been included. The methods and results are summarized concisely. The type of data analysis used to test the results should have been mentioned.

INTRODUCTION

Results of a study of home range of largemouth bass, *Micropterus salmoides,* in a 3.4 hectare pond indicated that bass that strayed from their normal home range were able to return to it (Lewis and Flickinger 1967). Also, a large percentage of largemouth bass overwintering in deeper water returned in spring to the same segment of shoreline that they occupied the previous summer. This behavior suggested some mode of homing, but in a large reservoir largemouth bass failed to home when displaced to other parts of the reservoir (Chappell 1974; Smith 1974). This contradiction suggested the presence of sun-compass and Y-axis orientation or both in largemouth bass.

Goodyear and Ferguson (1969) and Goodyear (1970) demonstrated Y-axis orientation, that is, movement in a direction perpendicular to the shore of their capture (Ferguson and Landreth 1966), in mosquitofish *(Gambusia affinis)* and starhead topminnow *(Fundulus notti).* They also showed that these species use sun cues or "sun-compass" orientation for Y axis orientation. Hasler et al. (1958), Groot (1965), and Hasler et al.

The first paragraph introduces background information on the homing and orientation behavior of the test organism. The first time the test organism in mentioned, its complete binomial is used; thereafter the common name is used. The first study cited showed that largemouth bass were capable of homing behavior, while the second two studies showed that artificial relocation of the bass interrupted their ability to orient. These two pieces of background information suggest that bass may use a sun-compass or Y-axis orientation–the subject of this study.

This paragraph shows both methods of reference citation: the name of the author in the sentence with the date in parentheses or both name and date in parentheses. The studies cited provide background information on sun-compass and Y-axis orientation found in other species of fish. The authors indicate that this type of study has not been done with large-mouth bass, which supports the significance of the study.

(1969) have demonstrated sun-compass orientation in pumpkinseed *(Lepomis gibbosus),* sockeye salmon (*Oncorhynchus nerka),* and white bass *(Morone chrysops),* respectively. Although other Centrarchidae have been tested, the largemouth bass has not been tested for sun-compass or Y-axis orientation.

The objective of this research was to determine whether sun-compass or Y-axis orientation or both are manifest in largemouth bass.

The last paragraph of the introduction specifically states the objective of the study. A brief mention of the procedures to be used and the significance of the investigation should have been made. The omission of the significance of the study in the ecology of the large-mouth bass presents a problem in the discussion section of this paper.

METHODS

Juvenile largemouth bass (5-10 cm, total length) were captured on 25 July, 27 September, and 1 October 1974, by seining along the NW and SE shorelines of a 1.56-hectare pond on the South Carolina Agricultural Experiment Station at Clemson, South Carolina. The fish were held and transported in covered, opaque containers until orientation trials were conducted.

The test arena, a plastic-lined wading pool with a diameter of 3 m and water in it to a depth of 25 cm, was located outside on a hilltop so that nothing but the sky was visible overhead. The release mechanism consisted of a 13-liter, wide-mouth glass jar with the bottom removed and four cords attached to the neck, passed over the wall of the pool, and secured by stakes outside the pool. The cords were located on the primary compass points. When placed in the center of the pool, the jar could be raised by applying tension to two or more of the cords. The top of the wall was marked at 30° intervals for reference points.

At the beginning of this section, the authors should have repeated the full scientific binomial for the test organism. Details on the size of fish, date, method, and location of capture are well written. The number of fish involved would be useful information. Note the use of metric units (no period following abbreviation) and the method of presenting dates (1 October 1974). The use of opaque containers is mentioned because it is significant to the study.

This paragraph provides a clear and concise description of the experimental apparatus. Note that fairly simple equipment can be used in an experiment.

With the jar resting on the bottom of the

These two paragraphs detail the experimental procedure followed. A reader could not only repeat the study, but has sufficient information to judge the worth of the experimental procedure. There has still been no mention of the

pool, the fish were placed in the jar, allowed 30 sec to orient, then released. Fish behavior was noted by observers stationed behind blinds attached to the pool wall at the four principal compass points.

Bass were tested in the morning and afternoon on the day of capture. Fish from each location were released as a group six times and individually once. The direction in which the fish swam from the jar and the point at which the fish first came within 15 cm of the pool wall were recorded. Individuals that did not approach the perimeter of the pool within 1 min were not scored, so observations within each trial were unequal.

Tests were conducted on clear to partly cloudy days (25 July and 1 October) and on a heavily overcast day (27 September).

Data were subjected to the Watson-Williams analysis for circular distribution (Zar 1974) to test for differences in response of fish from NW and SE edges of the pond. Fish were tested individually and as a group, and tested in the morning and afternoon on clear and overcast days.

RESULTS

Responses of fish when released in the test arena are shown in Figure 1. On clear to partly cloudy days the behavior of fish from one side of the pond was significantly different (P < 0.01) from that of fish from the opposite side. With regard to fish tested individually or in groups, only the fish from the NW, when tested under overcast skies, showed a significant difference in orientation. Morning and afternoon results were not significantly different from each other.

When the angles of all clear-day observations were combined, the resultant vectors (Fig. 2) indicated NW orientation (288°)

number of fish that were used.

The name and source of the statistical analysis used are provided, along with a general mention of the data that were tested. The last sentence is unclear. Are the authors indicating that the <u>data</u> for the <u>responses</u> of fish for those different trials and times were "tested" in the statistical analysis? Or, is the sentence merely redundant because what it states has been mentioned before in more detail?

A reference is made to the results illustrated in Figure 1, but no written description is provided. Figure 1 contains a great deal of information which the reader has to work hard to extract. The most important features of this figure should have been verbalized. The second sentence clearly states the result of the statistical test for the behavior of fish on clear to partly cloudy days. *P* < 0.01 indicates the relative confidence level for the test result-- there is less than a 1% chance that these results could have occurred by chance. The third sentence is not easy to understand. Perhaps this sentence is clearer: *Only the fish from the NW, when tested under overcast skies, showed a significant difference between their orientation when tested individually and their orientation when tested in groups (See Fig.1.b).* It is important that the reader understand exactly what data were compared.

This paragraph clearly describes the results shown in Figure 2. Notice that throughout the Results section, no interpretation or discussion of data is included.

for fish from the SE side of the pond and SE orientation (126°) for those from the NW side of the pond. On the overcast day the orientation of both groups was toward the SW; 227° and 216° for SE and NW groups, respectively.
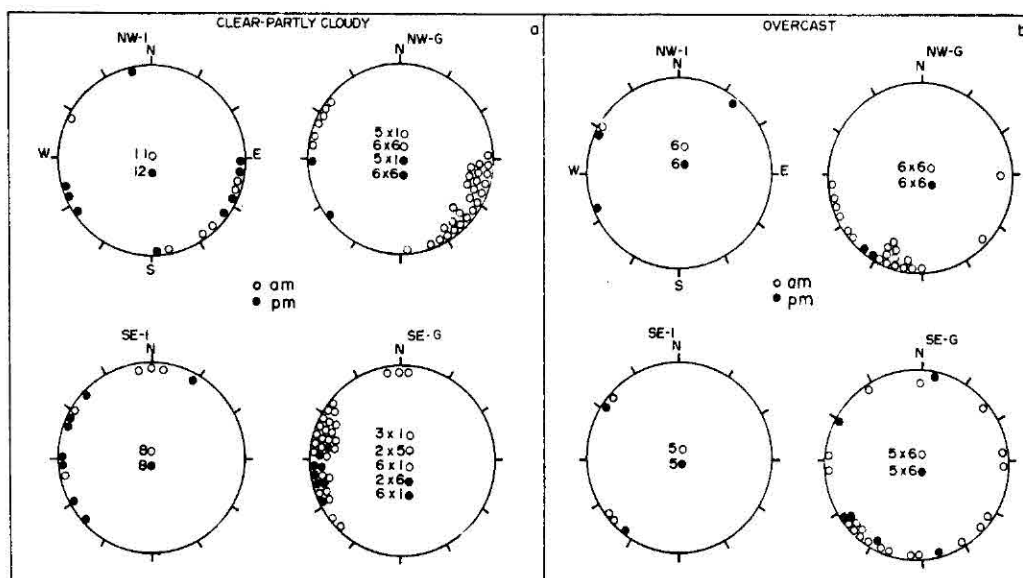

FIGURE 1.—*Directional responses in test arenas of juvenile largemouth bass from NW and SE natural shore lines under (a) clear to partly cloudy skies and (b) overcast skies. NW = fish from northwest shore of pond; SE = fish from southeast shore of pond. I = individual releases, G = group releases. The numbers of fish tested in morning or afternoon are indicated near the center of each diagram, for group releases, the first numeral is the number of fish in the group and the second numeral is the number of releases for that group. Fish that did not reach the perimeter within 1 min were not scored.*

Figure caption is placed <u>below</u> the figure. Each symbol and convention used is clearly described, although the reader must spend some time referring to the caption and diagrams in order to interpret the figure. All the data gathered in the study are presented in a highly condensed and diagrammatic form.
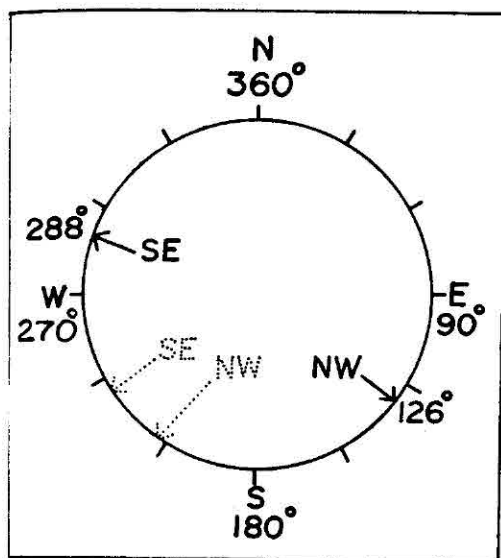
This figure clearly illustrates the major results of the study: on clear to partly cloudy days the fish from opposite shores orient in opposite directions, while fish did not orient in that manner on the overcast day. Tests were made on only one overcast day; the caption indicates the results apply to more than one.

FIGURE 2.—*The resultant angles for all responses in test arenas of juvenile largemouth bass from SE and NW shores of a pond and released on clear to partly cloudy days (solid arrows) and overcast days (broken arrows).*

DISCUSSION

Juvenile largemouth bass (5-10 cm, total length) appear to be oriented to move off-shore, although previous studies indicate that *Gambusia affinis* and *Fundulus notti* are oriented to move inshore (Goodyear and Ferguson 1969; Goodyear 1970). Apparently these species seek refuge from predation in the shallower water and "the behavior reflects intensity of predation" (Goodyear and Ferguson 1969).

The difference in orientation under sunny and overcast conditions is strong evidence for sun-compass orientation. The low number of scores from all fish released on the overcast day and the random responses of individually released bass on the overcast day reinforced the hypothesis that sun-compass was used by the fish tested. The group released under overcast skies demonstrated the strong social behavior (schooling tendency) inherent in juvenile largemouth bass. Further work should be conducted to determine whether there is a change associated with age or size in the manifestation of Y-axis orientation in largemouth bass and to explain its seeking refuge in deeper water.

LITERATURE CITED

CHAPPELL, J. A. 1974. Response of largemouth bass to thermal effluent from Oconee Nuclear Power Plant. M.S. Thesis. Clemson University, Clemson, S.C.

FERGUSON, D. E., AND H. F. LANDRETH. 1966. Celestial orientation of Fowler's toad, *Bulo fowleri*. Behaviour 24: 105-123.

GOODYEAR, C. P. 1970. Terrestrial and aquatic orientation in *the* starhead topminnow, *Fundulus notti*. Science 168:603-605.

The beginning of this sentence should stand alone and refer the reader to the results shown in the study: fish moved in a direction opposite to the compass direction of the shoreline from which they were captured. Reference is made to previously cited studies that had found opposite results for other species of fish. The reason for this behavior as proposed in those studies is given--to escape predation. Quotation marks were used because the authors used the exact wording of Goodyear and Ferguson. In general, quotations should not be necessary.

This paragraph clearly presents the results of the study as evidence for sun-compass orientation. No mention of schooling behavior was made in the Introduction or in the Results section. It is inappropriate to introduce new material such as this in the Discussion section. Also, the claim that the group released under overcast skies showed schooling tendency appears to apply only to the fish from the NW shoreline. The SE circle in Fig. 1 shows fish scattered at all compass points. The beginning of the last sentence suggests future studies to determine if Y-axis orientation (movement in a direction perpendicular to shore of capture) changes with age or size of the fish. The end of the sentence, however, makes too large a leap in implying that the movement offshore is to seek refuge in deeper water. No groundwork had been laid for this claim.

Lab. Instructor _____   **Name** _____

SCIENTIFIC WRITING EXCERCISE

*In the blank preceding each statement, classify the passage as being most appropriately included in the Introduction (**I**), Methods (**M**), Results (**R**), or Discussion (**D**) parts of a scientific paper.*

_____In this paper, I report on sexual differences in foraging sites, activity budgets, and foraging efficiencies of male and female Black-capped Chickadees (*Parus atricapillus*) in poplar (*Populus tremuloides*) woodlands.

_____The study area consisted of a 250-ha mosaic of poplar woodland interspersed with fields (20% of the area) and stands of willows of ≤4 ha.

_____Statistical differences (Kruskal-Wallis tests; a < .05) in the foraging heights among members of the same sex were found in five flocks, but no significant differences were found between foraging heights and intersexual dominance status (Table 2).

_____Males removed from experimental flocks were immediately brought to indoor cages, weighed, and provided with ad libitum sunflower seeds, water, and softbill mix (dried arthropods).

_____All data comparisons were evaluated with nonparametric statistical tests.

_____The removal experiment showed that the foraging sites of females were largely influenced by the presence of males, suggesting intersexual competition for winter resources within the flocks.

_____Table 1 shows the effect of darkness on the membrane potential, membrane resistance, and K^+ influx at pH 6 in APW ± 0.4 mM KCL.

_____The values for the membrane conductance (Figure 1) indicate that the constant current pump model is not appropriate for the *Chara* membrane.

_____Field experiments on intersexual niche partitioning are virtually absent from the literature, except for the work of Peters and Grubb (1983) on Downy Woodpeckers (*Picoides pubescens*).

_____The work of Lucas (1975) on *Nitella* sp. agrees with our data (Table 3) estimating the membrane potential in *Chara*.

_____The results were expressed as concentration of ATP in the cytoplasm, knowing that the ATP was in the cytoplasm (Miller AG, personal communication) and assuming that the cytoplasm is 5% of the cell volume.

Some of these passages were modified from papers by:

Desrochers A. 1989. Sex, dominance, and microhabitat use in wintering black-capped chickadees: a field experiment. Ecology 70(3):63-645.

Kiefer DW, Spanswick RM. 1979. Correlation of adenosine triphosphate levels in *Chara corallina* with the activity of the electrogenic pump. Plant Physiology 64:165-168.

For each passage in the following section, briefly discuss why the passage is not appropriate for the part of the scientific paper indicated.

Title

"The orientation of blowflies to two simultaneous light sources of varying intensities."

"   **Abstract**

"Our results on *Daphnia magna* orientation to red light (Table 1, Figure 2) indicate a high sensitivity to this color."

"   **Introduction**

"The data we collected on attraction to conspecifics in swordtails (*Xiphophorus maculatus*) does not support the view of fish school formation suggested by Burgess and Shaw (1979)."

"   **Methods**

"The distance under the coverslips was measured to be 0.25 mm (Figure 1). This may have prevented easy egress by planaria."

"To insure that all petri dishes are identically filled, measure 25 ml of pond water using a 100 ml Corning" graduated cylinder and pour it into the dish."

"All time measurements were written in tabular form using a no. 2 pencil in our lab notebooks and subjected to statistical analysis with the sign test."

"   **Results**

"The data presented in Table 3 supports our hypothesis that humidity is a more important stimulus to sowbugs than light and we therefore reject the null hypothesis (H_O) that time spent in the humid-light chamber does not differ from the time spent in the dry-dark chamber."

"See Table 1 showing the four rank sum tests we performed on the data showing number of sowbugs in the two chambers of the choice apparatus. All differences were significant at the a = 0.05 level."

"   **Discussion**

"Our results do not support our initial hypothesis."

APPENDIX 4—STATISTICAL REFERENCE

Jon C. Glase and Michael C. Runge

INTRODUCTION

Scientific investigations are concerned with determining if the predictions of research hypotheses are valid. Using either experimental or observational means, data are collected that test the predictions of a research hypothesis by allowing or not allowing rejection of the null hypothesis (H_O) which states that the data being compared do not differ. In order to decide if data support a certain research hypothesis, an objective procedure is required for rejecting or accepting H_O. To be objective, the procedure must be based **exclusively** on the evidence collected in the investigation and should not in any way reflect personal bias or interests. The scientist must be willing to accept the consequences of this procedure and any modifications it may require of the original research hypothesis. It was in the realm of statistics that scientists found methods for helping them describe their data (**descriptive statistics**) and make the necessary objective decisions based on them (**statistical testing**).

This appendix includes reference information on descriptive statistics and statistical testing, and detailed descriptions of tests used in conjunction with your laboratory work in BioG 1107-1108.

DESCRIPTIVE STATISTICS

In most scientific studies, the investigator is selecting a subset of "things" (measurements from organisms, cells, tissues, etc.) for detailed study from some larger **population** of the same "things". The subset is called a **sample** and the things composing the sample are called **replicates**. The investigator is actually interested in the larger population from which the sample came, but because of the impossibility (or desirability) of studying each member of the population, a sampling procedure must be used. Because the investigator wants to make inferences about the larger population from the sample, it is critical that the sample reflect in every way the larger population's characteristics. A sample is information about the population, and the sample size, usually expressed as **n**, represents the amount of information that has been collected.

After sample collection, the biologist is often faced with a large number of data whose significance is not obvious at first glance. Raw, unordered data (Table 1) are difficult to interpret, either in terms of estimating an average data value or the spread or variation of the data. To aid interpretation, a **data array** is constructed in which the data are ranked from the lowest to the highest value (Table 2). Data can also be grouped into **classes**, and the number of data values within each class, the **frequency**, tabulated (Table 3). The class width, called the **class interval**, is chosen arbitrarily but should fit the data properly. From a class list, it is possible to construct a **histogram** (bar graph) in which the width of each rectangle represents the class interval and the height represents the frequency of data values in each class (Figure 1). The histogram allows one to identify those classes which have higher frequencies than others and to estimate the general shape of the data distribution.

Table 1. Unordered weight data, in grams, of wild black-capped chickadees (*Parus atricapillus*) caught between 0800 and 1600 on 8 December 1969.

11.49, 10.85, 10.66, 10.89, 10.07, 11.19, 11.12, 12.69, 10.79, 11.37, 10.78, 9.93, 11.41, 10.81, 11.75, 12.21, 10.44, 10.34, 12.08, 9.86

Table 2. A data array of the chickadee weights, in grams, from Table 1 ordered from lowest to highest.

9.86, 9.93, 10.07, 10.34, 10.44, 10.66, 10.78, 10.79, 10.81, 10.85, 10.89, 11.12, 11.19, 11.37, 11.41, 11.49, 11.75, 12.08, 12.21, 12.69

Table 3. Chickadee weight data grouped into frequency classes.

Class Interval (g)	Frequency
9.51 - 10.00	2
10.01 - 10.50	3
10.51 - 11.00	6
11.01 - 11.50	5
11.51 - 12.00	1
12.01 - 12.50	2
12.51 - 13.00	1
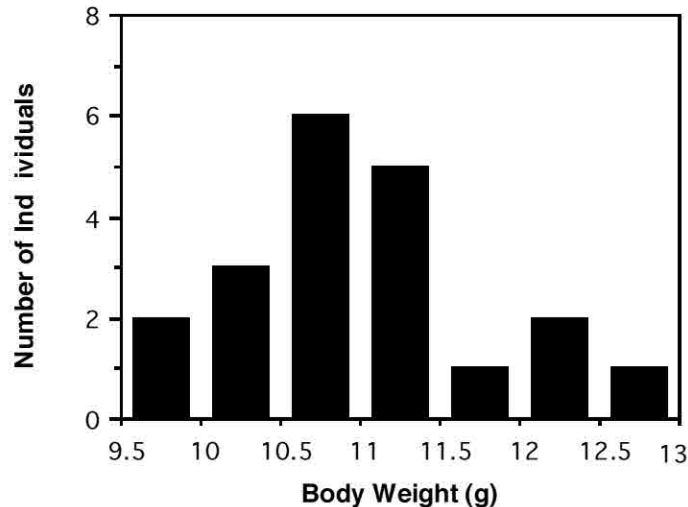
Figure 1. A histogram of chickadee weight data.

Mean, Variance, Standard Deviation

Two very useful and fundamental ways of describing your sample are to characterize the **central tendency** and **variation** shown by the data. The **mean** ($\overline{X}$) or arithmetic average is a measure of central tendency since it describes the point about which all data congregate. Mathematically it is:

Equation I:

$$\overline{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

where: X_i = individual data items

n = the number of data in the sample

Note: The sigma sign ($\sum$) means that you sum all of the data items (X_i) from i = 1 to i = n.

Two other measures of central tendency, the **median** and **mode**, are also useful in describing a normal distribution. The median is defined as the middle data value in the data array. If there were five data values in a sample (5, 7, 9, 11, 13), data value three (9) would be the median. The mode represents the most frequently occurring data value and can be easily observed in a data array. If the sample has two data values which occur with the greatest frequency, the distribution is termed **bimodal**.

One must have not only a good estimate of central tendency to describe a distribution, but also an estimate of data variation, or scatter. The simplest estimate of replicate variation is the **range** of data values in an array. The range is obtained by subtracting the smallest data value from the largest data value. Other important estimates of dispersion are the **variance (S^2)** and the **standard deviation (S)**, the square root of the variance. To obtain the variance of a sample, subtract the mean from each data value, square this difference (deviation), sum the individual squared deviations and divide the total of the squared deviations by n-1. Mathematically, it is:

Equation II:

$$S^2 = \frac{\sum_{i=1}^{n} (X_i - \bar{X})^2}{n-1}$$

The standard deviation is simply the square root of the variance. Whenever a sample contains a large number of data values, Equation II becomes cumbersome. An equivalent formula (Equation III) is designed for use with electronic calculators.

Equation III:

$$S^2 = \frac{\left(\sum_{i=1}^{n} X_i^2 \right) - \frac{\left(\sum_{i=1}^{n} X_i \right)^2}{n}}{n-1}$$

Although at first it appears more difficult, the calculator automatically stores, in memory

$$\left(\sum_{i=1}^{n} X_i^2 \right) \text{ and } \left(\sum_{i=1}^{n} X_i \right)$$

The rest of the manipulations can be easily performed on the calculator.

Why do we divide by n-1 instead of n in obtaining the variance? This is understandable if we recall that a sample represents **information** about a larger population. Since n is the size of our sample, it quantifies the magnitude of this information. The larger that n is the greater is the information that we have about the population. With all other things being equal, a larger sample should have a larger amount of variation than a smaller sample. This is because only with a large sample will obtaining extreme population members be probable. It is for this reason that the sample size acts as the divisor in calculating the variance. But, why do we divide by n-1 and not n? Simply because we do not want to pretend that we have more information than we actually have. For example, imagine that you are in a dark room trying to put on a pair of shoes. You pick up one shoe and discover that it is the shoe fitting your right foot. The identity (right or left) of the other shoe is immediately known. Similarly, if you know what each of the sample values (X_i) is except the last one and you know $\sum X_i$, then you automatically know what the last data value must be. Thus, to indicate the amount of real information that our sample represents from the larger population, we divide by n-1 and not n.

The example that follows should help clear up any difficulties you have had in understanding mean, variance, and standard deviation. The data are the previously presented body weights of wild-caught chickadees (Tables 1-3).

i	X_i (g)	$X_i - \overline{X}$	$(X_i - \overline{X})^2$
1	9.86	−1.18	1.39
2	9.93	−1.11	1.23
3	10.07	−0.97	0.94
4	10.34	−0.70	0.49
5	10.44	−0.60	0.36
6	10.66	−0.38	0.14
7	10.78	−0.26	0.07
8	10.79	−0.25	0.06
9	10.81	−0.23	0.05
10	10.85	−0.19	0.04
11	10.89	−0.15	0.02
12	11.12	0.08	0.01
13	11.19	0.15	0.02
14	11.37	0.33	0.11
15	11.41	0.37	0.14
16	11.49	0.45	0.20
17	11.75	0.71	0.50
18	12.08	1.04	1.08
19	12.21	1.17	1.37
20	12.69	1.65	2.72
	220.73		10.94

<u>sample size</u> (n) = 20

$$\text{mean } (\overline{X}) = \frac{\sum\limits_{i=1}^{20} X_i}{20} = \frac{220.73}{20} = 11.04 \text{ g}$$

$$\text{variance } (s^2) = \frac{\sum\limits_{i=1}^{20} (X_i - \overline{X})^2}{n-1} = \frac{10.94}{19} = 0.58$$

$$\text{standard deviation } (S) = \sqrt{s^2} = \sqrt{0.58} = 0.76 \text{ g}$$

<u>variance calculation </u>(calculator formula)

$$s^2 = \frac{\left(\sum\limits_{i=1}^{20} X_i^2\right) - \dfrac{\left(\sum\limits_{i=1}^{20} X_i\right)^2}{n}}{n-1} = \frac{2447.04 - \dfrac{(220.73)^2}{20}}{19} = \frac{2447.04 - 2436.09}{19} =$$

$$\frac{10.94}{19} = 0.58$$

$$S = \sqrt{0.58} = 0.76 \text{ g}$$

If your sample size includes <u>five or more</u> replicates, calculation of a mean and standard deviation may be meaningful. In scientific reports the mean and standard deviation are typically presented in the format **mean (± standard deviation)** or, for the above example, 11.04 (±0.76) grams. Notice that the standard deviation is expressed in the same units as the mean whose variation it represents.

Median, Quartiles, and Interquartile Range

There are situations where the mean and the standard deviation can be misleading measures of central tendency and variation, respectively. For example, consider household income in 1989 in Tompkins County (as measured in the 1990 Census). The **mean** household income was approximately $36700, but less than 39% of the households had income greater than the mean. This is an example of a **skewed distribution** in that there are more data points on one side of the mean than the other. Household income is skewed because there are some households with very high incomes and these values have a considerable influence in raising the overall mean.

On the other hand, the **median** household income was $27742. By definition, 50% of the data points fall above the median, and 50% below, whether the distribution is skewed or symmetric. In this case, economists consider the median to be a much better measure of the "typical" household income.

As noted earlier, the median is the "middle" value in the ordered data series. An easy formula for which value this is in the ordered series is:

$$(n + 1)/2$$

where n is the number of values in the data set. For example, in the chickadee weight data set, Table 2 (page A37), there are 20 ordered data values, therefore the median is the $(20 + 1)/2 = 10.5^{th}$ value. What does it mean to have an ordered statistic of 10.5? It means that the median is the value which is 50% of the way between the 10th and 11th points. In the chickadee data, the 10th value is 10.85 and the 11th value is 10.89. Half-way between these is 10.87, so the median chickadee weight is 10.87 g.

There are several descriptive statistics related to the median, called the first quartile and the third quartile. The **first quartile** is the median of the lower half of the data, and the **third quartile** is the median of the upper half of the data. To find the first quartile, first rank the data. Multiply the sample size by 0.25. If this number is an integer, add 0.5. If the number is a decimal, round it up to the nearest integer. Now, find the data value in this position. If the position ends in 0.5, take the midpoint between the two adjacent values (as above). The third quartile is found in an analogous manner, except instead multiply the sample size by 0.75.

For example, in the chickadee data set, the first quartile would have an ordered statistic of 5.5 (since 20 × 0.25 = 5, which is an integer, so add 0.5), that is, it is the value which is halfway between the 5th and 6th values, which are 10.44 and 10.66, respectively. Thus, the first quartile is 10.55. Likewise the ordered statistic of the third quartile is 15.5 (20 × 0.75 = 15; 15 + 0.5 = 15.5), that is halfway between the 15th and 16th values, which are 11.41 and 11.49, respectively. Thus, the third quartile is 11.45.

Together, the first and third quartiles give a measure of how spread out the data is. The difference between them is called the **interquartile range (IQR)**. This can be used instead of the standard deviation as a measure of the variation in the data. The interquartile range for the chickadee masses is 0.90 grams (11.45 − 10.55). Notice that one way to understand the first quartile is as the point *below* which 25% of the data fall. Likewise the third quartile is the point *above* which 25% of the data fall. Thus, the middle 50% of the data fall between the first and third quartiles, and the interquartile range expresses how close together this middle 50% is. In the chickadee data, we would say that 50% of the chickadee masses are within 0.90 g of each other.

The median and the interquartile range are analogous to the mean and the standard deviation, in that they are measures of the central tendency and the variation, respectively. The advantage of the median and interquartile range is that these statistics are **robust**, that is, they are not sensitive to extreme values.

Consider the data:

        1, 4, 7, 15, 16

for which the mean is 8.6 and the median is 7. If we change the last point to 87, the mean becomes 22.8, but the median stays the same. A change in just one value can have a very large effect on the mean, but no effect on the median. A similar effect is seen in the interquartile range. This robust feature is advantageous when the data shows a skewed distribution, or when there may be a small number of measurement errors in the data.

Normal Curve

Frequently, a sample taken from some larger population when plotted as frequency versus some sample measurement produces a bell-shaped, or **normal curve** (Figure 2).
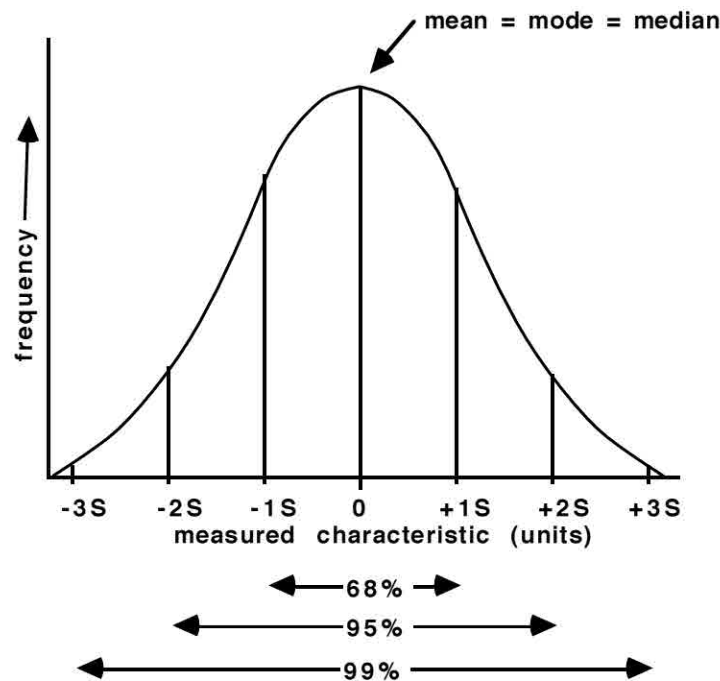
Figure 2. A normal, bell-shaped curve showing the relationship between standard deviation and area. The area under any part of the curve represents the fraction of the population which exhibits the range of the measured characteristic indicated on the abscissa. The area between -1S and +1S is 68%, that between -2S and +2S is 95%, and that between -3S and +3S is 99% of the total area under the curve.

Sample data showing this type of relationship are said to be **normally distributed** and possess certain useful quantitative properties. These properties are:

1.   the curve described by a normal distribution is symmetrical (bell-shaped) with the mean, median, and mode equal to each other;

2.   the area beneath any segment of the curve is proportional to the number of observations associated with that segment;

3.   the standard deviation is related to the normal curve such that ± 1S from the mean encompasses 68% of the data, ±2S = 95%, and ±3S = 99% of these data (see Figure 2).

Point three is particularly valuable because it allows you to use the standard deviation in assessing the amount of variation associated with a given sample. That is, a sample with a large S has greater variation among the replicates than another sample with a smaller S. Also, in the example examining the weight of birds in a sample of wild chickadees caught between 0800 and 1600, 8 December 1969 (see page A37), we would estimate that 68% of the total population from which the sample was taken should be birds with weights within ±0.76 grams of the mean weight (11.04 g). Similarly, 95% of the population members should have weights within ± 2 × 0.76 or 1.52 grams of the mean weight.

Error Bars

A useful way to graphically depict the amount of variation associated with several means is shown in Figure 3, which shows the mean numbers of limpets (*Diodora aspera*) per square meter from four locations, based on ten yearly samples taken during the period 1961-1970. Error bars are used to show the magnitude of each mean's standard deviation. In constructing error bars the vertical line above the mean equals +1.0 standard deviation; the vertical line below the mean equals -1.0 standard deviation. The mean for sample one equals 39 individuals/m^2 and its standard deviation is 17 individuals/m^2. Thus, about 68% of all samples included in the mean for Lundy Bay should have between 22 and 56 limpets per m^2. We can see that although Lundy Bay and Angle Bay have about the same limpet density, there is about one half as much variation associated with samples from Angle Bay; the standard deviation of the mean for Angle Bay is about 8 or half as much as the standard deviation of the mean for Lundy Bay. Also, the amount of overlap in the error bars of means is a good way to visually compare the amount of overlap in the data values of the samples. If the error bars overlap extensively, as in the means for Lundy Bay, Angle Bay and Black Point, the means are probably not different. If the error bars of means do not overlap, as for the first three locations compared with Pie Island, or Angle Bay verses Black Point, then the means are probably different. However, statistical testing is required to objectively decide if apparent differences shown by this technique are large enough to be considered statistically significant, as discussed in the next section.
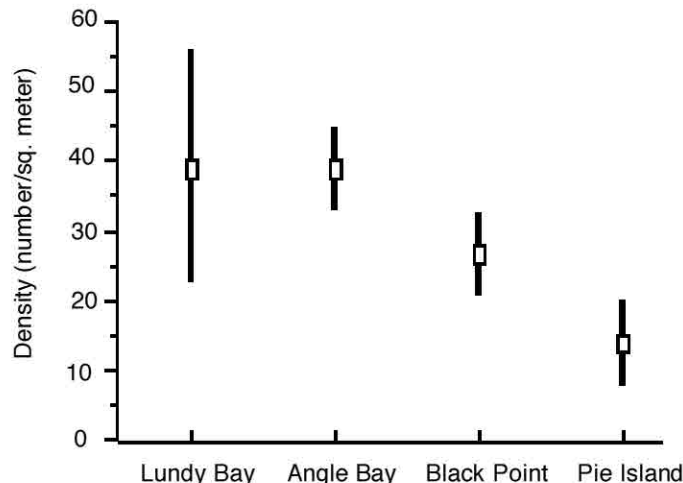
Figure 3. Mean limpet (*Diodora aspera*)densities (with error bars) from samples taken during the years 1961-1970 in four locations on the east coast of England.
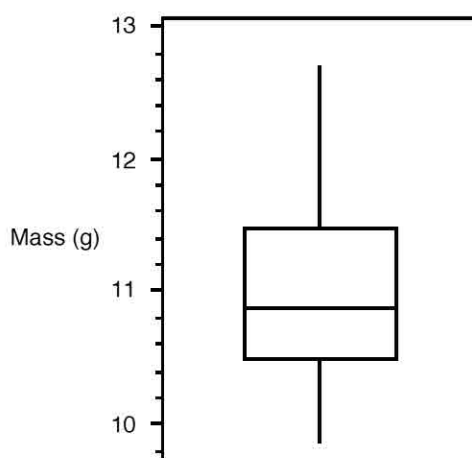
Boxplots

Another way to graphically depict the amount of variation in one or more samples is to use a **boxplot**. Figure 4 shows a boxplot of the chickadee weight data. To draw a boxplot, first draw a linear scale on the vertical, y–axis that spans the entire range of the data. Draw horizontal lines from this at the first quartile, the median, and the third quartile. Draw two vertical lines to close this box. Calculate the interquartile range (IQR), multiply by 1.5 and add this to the third quartile. Identify the greatest data value that is less than this value and draw a vertical line from the box up to it. Any points greater than this value

are called **outliers** and should be indicated individually with asterisks. Likewise, subtract 1.5 × IQR from the first quartile. Identify the <u>smallest</u> data value that is <u>greater</u> than this value and draw a vertical line from the box down to it. Any points lower than this value are outliers.

A quick look at a boxplot conveys quite a bit of information. The central horizontal line shows the median, that is, the central tendency of the data. The box encompasses the middle 50% of the data. The "whiskers" extend out to include all the "reasonable" data, and each individual outlier is shown with an asterisk.

Several boxplots placed next to each other on the same scale can be used to compare different treatments or different categories. For example, Figure 5 shows a comparison of the wing length between male and female Black-capped Chickadees. This graph suggests that males have longer wings than females, and that the variation in length of the wings in males is greater than in females (as evidenced by the larger IQR, that is, a larger box). No outliers are present in these data.

Figure 4. Boxplot of weight data, in grams, of wild Black-capped Chickadees (*Parus atricapillus*) caught between 0800 and 1600 on 8 December 1969. There are no outliers in this dataset.
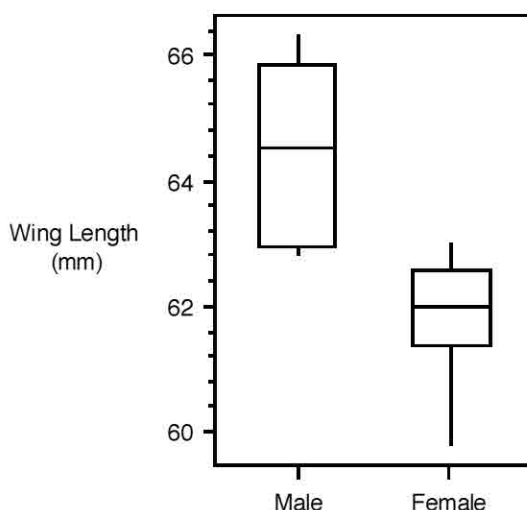
Figure 5. Boxplot of wing length, in millimeters, for male and female Black-capped Chickadees. The sample size for each sex is 10.

How do you know when to use the mean, standard deviation, and error bars or the median, interquartile range, and boxplots to represent your data? Generally, if the mean and median are similar, there are no outliers, and the sample size is at least 6–7, you should use means and standard deviations. Otherwise, use medians and IQR's.

STATISTICAL TESTING

In an experimental test of hypothesis you compare one or more experimental groups with a control group in order to determine if any differences between the groups are significant. In an observational test of hypothesis you compare the new observations you made with the observations you expected based on your research hypothesis. In both cases, statistical testing uses your sample data to determine the probability that the same results (your data) could have been obtained purely by chance. The probability that the same results were produced by chance, under the assumption of the null hypothesis, is called the **alpha level (α)**. The four steps of statistical testing are the same for all statistical tests.

Step One: Calculate a Test Statistic. Most statistical tests are a series of data manipulations which lead to the calculation of a number called the test statistic. The test statistic quantifies the differences observed in the different data groups. The manner in which the test statistic is calculated depends on the specific test used. In all cases the test statistic is compared with another number called the tabular statistic, usually found in the appropriate statistical table for the test you are using.
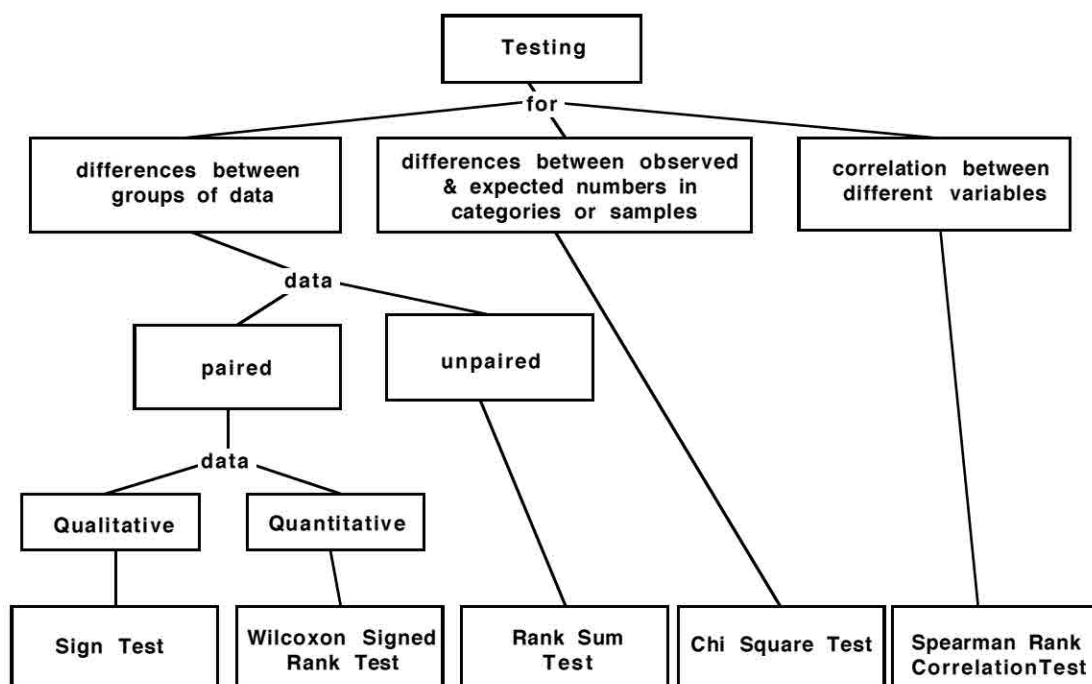
Step Two: Select a Tabular Statistic. The tabular statistic represents the minimum amount of difference needed at some specified α level in order to reject H_o. In statistical tables, tabular statistics are usually indexed according to sample size and alpha level (α).

Step Three: Comparison. Compare your test statistic value, as calculated from your actual data, with the tabular statistic value at a certain alpha level and sample size to determine whether or not you can reject H_o. In some tests you can reject H_o at a given alpha level if the test statistic is greater than or equal to the tabular statistic; in other tests the test statistic must be smaller than or equal to the tabular statistic for you to reject H_o. The relationship between test statistic and tabular statistic needed for rejection of H_o depends on the way the test quantifies the sample variation, and is clearly stated in the test procedure.

Step Four: Decision. It is important to note that the decision to reject or not reject the H_o and any consequent revision of the research hypothesis follows objectively from the statistical testing procedure of the sample data included in the study. Also, unlike most commonly made decisions, a decision based on statistical testing has a probability value associated with it. The alpha level represents the probability of rejecting the null hypothesis, when in fact the null hypothesis is true. If your results allow rejection of H_o at the 0.05 α level, then if the null hypothesis were true, a test statistic as extreme or more extreme than the one you observed would occur only 5% of the time. The logic of rejecting H_o is as follows: if H_o is true, then you just witnessed a rare (5% chance) event; you are unwilling to believe that you witnessed such a rare event; thus, you reject H_o. If a comparison of the test and tabular statistics allows rejection of H_o at α = 0.05, you can say that the differences between your groups are **statistically significant**. If your results allow rejection at the 0.01 α level, then you are saying that under the null hypothesis, this would be a very rare event (1 % chance), which you are unwilling to accept, so you reject H_o. If a comparison of the test and tabular statistics allow rejection of H_o at α = 0.01, you can say that the differences between your groups are **highly statistically significant**.

PARAMETRIC AND NONPARAMETRIC STATISTICAL TESTS

A **parameter** is a quantifiable characteristic of a population. Parameters such as the mean, variance, and standard deviation characterize populations with a normal distribution. Parametric statistical tests make assumptions about the underlying characteristics of the population from which samples have been obtained. For example, many parametric tests assume that the underlying population shows a normal distribution. Nonparametric tests make no such assumptions. Most of the statistical tests presented in this appendix are nonparametric. The main advantage of nonparametric tests is that small sample sizes are permissible. Usually if your sample includes a minimum of 5 to 6 replicates, you can use a nonparametric test. Most parametric tests require two or three times as many replicates before being used validly. Smaller sample size is possible with nonparametric tests because the collected data are usually converted to categories or ranks (see below). As a result, nonparametric tests are not as powerful as parametric tests, which work with the data values directly. Five statistical tests are presented in this appendix. Each is designed for certain kinds of data and investigative designs. Criteria useful in selecting the correct statistical test are shown in the following flow chart.

```
                              ┌──────────────┐
                              │   Testing    │
                              └──────┬───────┘
                                  ── for ──
          ┌───────────────────┐ ┌───────────────────────┐ ┌──────────────────┐
          │ differences between│ │ differences between    │ │ correlation between│
          │  groups of data    │ │ observed & expected    │ │ different variables│
          │                    │ │ numbers in categories  │ │                   │
          └─────────┬──────────┘ │ or samples             │ └─────────┬────────┘
                 ── data ──      └───────────────────────┘
              ┌─────────┐   ┌──────────┐
              │ paired  │   │ unpaired │
              └────┬────┘   └──────────┘
                ── data ──
         ┌────────────┐ ┌──────────────┐
         │ Qualitative│ │ Quantitative │
         └─────┬──────┘ └──────┬───────┘

  ┌───────────┐ ┌──────────────┐ ┌───────────┐ ┌────────────────┐ ┌────────────────┐
  │ Sign Test │ │Wilcoxon Signed│ │ Rank Sum │ │ Chi Square Test│ │ Spearman Rank  │
  │           │ │ Rank Test     │ │  Test     │ │                │ │ CorrelationTest│
  └───────────┘ └──────────────┘ └───────────┘ └────────────────┘ └────────────────┘
```

TYPES OF DATA

Typically in nonparametric statistics, data must be in the form of either discrete categories or ranks. In some studies the data will be collected in either of these two forms. Otherwise, the procedure you use in calculating the test statistic transforms your data into the correct form. These data types and several other terms useful in deciding which statistical test is appropriate in a certain investigative design are discussed below.

Category Data. Using sample measurements or characteristics, numerical data can be classified as belonging to two or more discrete **categories**. For example, in a genetics study we may measure pea stems, classifying them, based on previously established criteria, as either short or long. In a study of the social interactions of a particular bird species, individuals can be classified as either dominant or submissive. Sometimes the actual data collected will already be in category form. For example, in a study of the lethal effects of a certain insecticide on house flies, the data are the number of flies classified as either alive or dead.

Ranked Data. In ranking, numerical data are arranged according to magnitude (i.e., smallest to largest or the reverse). For example, in our measurements of **n** pea stems, plants could be ranked from 1 to **n** according to the increasing length of their stems. The data on dominant-submissive behavior could be used to rank individuals from least dominant (rank 1) to most dominant (last rank).

Paired and Unpaired Data. In a "classical" experimental test of hypothesis, an investigator applies a specified treatment to a group. The response of this experimental group is then compared to a control group that was not exposed to the treatment. If the treatment has had an effect on the parameter measured, the data from the two groups should be statistically different. This type of data is called **unpaired** data. In an attempt to reduce the effects of extraneous variation in the experimental test of hypothesis, a paired experimental design can be used. This design type produces **paired** data. For example, by making pretreatment and post-treatment measurements on the same replicate, it can serve as its own control. This powerful design is called **self-pairing**. If self-pairing is not appropriate, one can match individuals according to size, age, or some other relevant criteria and randomly assign members of matched pairs to either the experimental or control group. Subsequent statistical testing looks for difference with respect to matched pairs. The matching design is only as good as your ability to match individuals in a meaningful way. This problem is avoided when self-pairing is used since no more precise pairing is possible than when the same individual acts as its own control.

Testing for Differences or Correlation. In addition to trying to determine if data groups differ, scientists are frequently interested in determining if two sample variables are in any way related. If two variables vary in some regular way with respect to each other they are said to be **correlated**. Correlation can be both **positive** (as one variable increases so does the other) or **negative** (a one increases the other decreases). Statistical tests of correlation specify the probability that a correlation exists between two variables. The test statistic produced by most tests of correlation also indicates the nature (positive or negative) and degree of association between the two variables.

STATISTICAL TESTS AND TABLES

Sign Test

The name of this test is derived from the fact that plus and minus signs are used in place of quantitative data. It is designed for use with data from an experiment in which **pairing** has been done. In the sign test, the test statistic is obtained by determining the signs of the differences between paired data values. The magnitude of the differences is ignored. Because of its simplicity it is not an appropriate test if the number of pairs (n) is less than 10. As n approaches 20, the sign test becomes more efficient. Use the sign test if your sample size is fairly large and if you think that the magnitude of differences between paired data values is not meaningful. If the magnitude of differences between matched pairs (or self-pairing) is meaningful, use the more powerful Wilcoxon signed rank test.

In the sign test, if no real differences exist between control and experimental pair members, one would expect equal number of positive and negative signs associated with the algebraic subtraction of the paired values; $\Sigma(+) = \Sigma(-)$. The sign test examines the deviation of the number of positive or negative signs from the value expected under the H_o, that is, one half of the sample size, and specifies the probability of obtaining this deviation if H_o is true. The test statistic is the total number of occurrences of the sign appearing with the lower frequency in the data. The test statistic is calculated by assigning a plus sign if the experimental value is larger than the control value, and a negative sign if the control value is larger than the experimental value. If experimental and control data values are equal (tied) for a pair, the pair must be excluded and n reduced appropriately. The tabular statistic is the maximum number of like signs of the lower frequency allowable at the specified alpha level. Table 4 contains the tabular statistic values indexed according to the number of pairs (n) in the sample, and $\alpha = 0.01$ or 0.05. If the test statistic is equal to or less than the tabular statistic, reject H_o. If the test statistic is greater than the tabular statistic, you cannot reject H_o. See the following example.

Example: An investigator was interested in determining if earthworms can locomote more rapidly on rough surfaces than on smooth surfaces. Two test arenas were utilized, each consisting of a 10-centimeter circle drawn either on a plate of glass backed with a sheet of balsa wood, or on balsa wood only. Worms were selected from those captured at night and matched according to overall body length. Simultaneously, each matched pair was placed in the center of the two test arenas and the amount of time required for each worm to move completely out of the circle was measured with a stop watch. Sixteen pairs (n=16) were tested in this manner. The rough-surface group was arbitrarily considered the control. The data and the statistical testing and conclusions follow:

Time Required to Leave Circle (sec.)

Pair	Rough Surface	Smooth Surface	Sign
1	10	8	-
2	14	9	-
3	5	11	+
4	6	15	+
5	10	16	+
6	30	7	-
7	11	30	+
8	15	16	+
9	38	42	+
10	19	22	+
11	14	40	+
12	18	5	-
13	28	20	-
14	8	46	+
15	17	19	+
16	10	33	+

H_o: Σ (+) does not differ signif. from Σ (-)   From the Data: $\Sigma(+) = 11$

H_a: Σ (+) does differ signif. from Σ (-) $\Sigma (-) = 5$

                                                                                                                        n = 16

Test Statistic = 5

Tabular Statistic (α = .05, n= 16) = 3 (Table 4)

Test Statistic (5) > Tabular Statistic (3); therefore, H_o cannot be rejected

Table 4. Numbers of like signs of the lower frequency allowed for significance at either α = .01 or .05 in the sign test.

No. of Pairs	α .01	.05	No. of Pairs	α .01	.05
5	----	----	13	1	2
6	----	0	14	1	2
7	----	0	15	2	3
8	0	0	16	2	3
9	0	1	17	2	4
10	0	1	18	3	4
11	0	1	19	3	4
12	1	2	20	3	5

If the number of paired observations (n) is greater than 20, a quantity (Z) must be calculated. Z is a factor based on a normal curve and gives us an acceptable approximation to use in a sign test for large sample size.

$$Z = \frac{(x \pm 0.5) - n/2}{0.5 \sqrt{n}}$$

where: x = number of (+) signs
n = sample size
use (x + 0.5)   if x < n/2
use (x - 0.5)   if x > n/2

If the absolute value of the resultant Z ≥ 1.96, there is a significant difference at α = 0.05.
If the absolute value of the resultant Z ≥ 2.58, there is a significant difference at α = 0.01.

Wilcoxon Signed Rank Test

This test improves upon the sign test by utilizing the magnitude as well as the direction of the differences between underlined paired observations. This test gives more weight to a pair with a larger difference between the two conditions than to a pair with a smaller difference. With a larger sample size (n ≥10) the efficiency of the Wilcoxon test increases greatly. The Wilcoxon test is more efficient than the sign test and should be used whenever information on the magnitude of the differences is meaningful.

Suppose that we are conducting a paired experiment to compare treatments A and B. Under the null hypothesis the effects of A and B are equivalent (H_o: effect of A does not differ significantly from effect of B). If we algebraically subtract the paired observations, theoretically we would expect to have an equal number of positive and negative differences. Also, the sum of the differences should equal zero. Under the same conditions, if we were to rank these differences we would expect the sum of the positive ranks to equal the sum of the negative ranks. We can therefore reject H_o if the sum of the ranks for either the positive or negative differences is small enough to be a significant deviation from equal sums of the ranks. The **test statistic** (T) is the smaller of the two rank sums (+ or -) and is computed according to the following procedure:

1.   Subtract the numbers associated with each pair of data values in a consistent order.

2.   Rank the absolute values (without regard to sign) of the differences (d) between the pairs of data values.
        a.   Smallest difference = rank 1.
        b.   If both values are the same (i.e., the difference = 0), disregard this pair in the analysis and reduce n appropriately.
        c.   If two or more pairs exhibit the same difference, assign them the average of the ranks which would have been assigned if the differences had resulted in sequential ranks (see example below).

3.   Assign to the ranks the signs of the original differences (+ or -).

4.   Compute the sum of the negative ranks (T-) and the sum of the positive ranks (T+).

5.   The **test statistic** is the absolute value of the smaller sum (T+ or T-). Compare the test statistic with the tabular statistic values in Table 5 (indexed according to sample size and α equals .05 or .01). **The tabular statistic is the largest value for T allowable in order to reject H_o.** Therefore, if the test statistic is equal to or less than the tabular statistic, reject H_o and assume that significant differences exist between the pairs. If the test statistic is greater than the tabular statistic, H_o cannot be rejected and this indicates that no significant differences exist between the data pairs.

Example: An investigator is interested in determining if brine shrimp show a difference in activity in bright or dim light. Activity is expressed as the number of seconds spent swimming in a four-minute test period. A self-pairing experimental design is used in which 10 brine shrimp are individually tested, first illuminated with dim light and then with bright light. The data and a statistical analysis of them using the Wilcoxon signed rank test follow:

Individual	Activity (seconds swimming) in:		d	Rank of Absolute Value of d
	Dim Light	Bright Light		
1	68	122	54	8
2	106	116	10	1.5
3	200	220	20	3
4	44	76	32	6.5
5	155	165	10	1.5
6	70	70	0	–
7	180	212	32	6.5
8	90	60	-30	(-)5
9	60	120	60	9
10	63	91	28	4

In the example above, data values in the "Dim light" column were subtracted from data values in the "Bright light" column because this gave more positive values in the d (difference) column.

However, subtraction of data values in the "Bright light" column from data values in "Dim light" column would be equally appropriate, if it is done consistently for all data pairs.

<u>Note</u>:

1. Data values for individual 6 are identical so these data are not included and n is reduced by 1.

2. Data pairs from individuals 2 and 5 have identical d's and are tied for ranks 1 and 2. Their ranks are determined by adding the sequential ranks and dividing by the number of tied pairs; (1 + 2)/2 = 1.5. Note that data pair 3 has the next largest d and is given the rank 3.

3. Similarly, d's for data pairs 4 and 7 are tied for ranks 6 and 7. Their ranks equal 6.5; (6 + 7)/2.

H_o: T- does not differ signif. from T+   i.e., light intensity does not affect brine shrimp activity

H_a: T- does differ signif. from T+   i.e., light intensity does affect brine shrimp activity

<u>From the Data</u>:   T+ = 40
T- = -5
n = 9

Test Statistic = 5
Tabular Statistic ($\alpha = .05$, n = 9) = 5
Test Statistic (5) = Tabular Statistic (5); therefore, reject H_o and accept H_a

Table 5*. Wilcoxon Signed Rank: largest values for T allowable to reject H_o at $\alpha = 0.05$ and $\alpha = 0.01$.

Number of pairs	0.05 level	0.01 level
6	0	–
7	2	–
8	3	0
9	5	1
10	8	3
11	11	5
12	14	7
13	17	10
14	21	13
15	25	16
16	30	19
17	35	23
18	40	28
19	46	32
20	52	37

*Adapted from Snedecor and Cochran, 1989. P.470.

If the number of paired observations (n) exceeds 16 you must calculate the factor Z which allows an approximation of the normal curve. The Z factor is calculated as follows:

$$Z = \frac{\frac{n(n+1)}{4} - T - 0.5}{\sqrt{\frac{n(n+1)(2n+1)}{24}}}$$

where: n = number of pairs
T = test statistic (absolute value of the smaller sum; T- or T+)

If the value of Z ≥ 1.96, we can assume that significant differences exist at $\alpha = 0.05$.
If the value of Z ≥ 2.58, we can assume that significant differences exist at $\alpha = 0.01$.

Rank Sum (Mann-Whitney) Test

As with the Wilcoxon signed rank test, the rank sum test converts primary data into ranks in order to obtain the test statistic. It is designed to determine if two samples are significantly different from each other. The two samples could be experimental and control groups in an experimental test of hypothesis or, as in the following example, two randomly selected groups in a nonexperimental test of hypothesis. The sample size need not be the same for both groups. This test should be used with underlined data. The following procedure is used in calculating the test statistic:

1. Rank the combined data values from both groups sequentially. Note: if the two sample sizes are not equal ($n_1 \neq n_2$) rank the data values (either smallest to largest or largest to smallest) so that the group with the smaller sample size has the smallest rank sum (see below).
2. Sum the ranks of each sample separately (T_1 and T_2).
3. The **test statistic** is the smaller of the two rank sums.
4. Compare the test statistic with the value of the tabular statistic (Table 6) at the appropriate sample sizes, and alpha levels (.05 or .01). The tabular statistic is the largest value for the smaller rank sum allowable in order to reject H_0. In using the table when sample sizes are unequal, let the smaller sample size equal n_1 and the larger, n_{22}.
5. If the test statistic is equal to or less than the tabular statistic you can reject H_0. If the test statistic is greater than the tabular statistic, you cannot reject H_0.
6. Data which tie for two or more ranks are given average ranks as calculated in the Wilcoxon signed rank test.

Example: In order to determine if a size difference exists between male and female black-capped chickadees, an investigator obtains wing length measurements for both sexes from birds of known sex. The following data on 10 adult male and 10 adult female chickadees illustrate the use of the rank sum test in determining if two groups are significantly different.

Wing Length (mm)

Rank	Male	Female	Rank
14	63.8	62.5	8
17.5	65.8	62.0	6
10	62.8	61.5	3.5
17.5	65.8	62.0	6
12.5	63.0	62.0	6
16	65.0	63.0	12.5
19	66.0	61.0	2
15	64.0	62.8	10
10	62.8	61.5	3.5
20	66.3	59.8	1

$T_1 = 151.5$ $T_2 = 58.5$
$n_1 = 10$ $n_2 = 10$

H_o: T_1 does not differ signif. from T_2; there is no difference in the wing lengths of males and females

H_a: T_1 does differ signif. from T_2; there is a difference in the wing lengths of males and females

Test Statistic = T_2 = 58.5

Tabular Statistic (n_1 = 10, n_2 = 10; α = .01) = 71

Test Statistic (58.5) < Tabular Statistic (71); therefore, reject H_o and assume a highly significant difference between groups

If the two sample sizes are larger than the table's values, calculate an approximation to the normal curve as follows:

$$Z = \frac{|\mu - T| - 0.5}{\sigma}$$

where: T = the smaller rank sum (T_1 or T_2)

$$\mu = \frac{n_1(n_1 + n_2 + 1)}{2}$$

n_1 = smaller sample size
n_2 = larger sample size

$$\sigma = \sqrt{\frac{n_2 \bullet \mu}{6}}$$

Then, if the value of Z ≥ 1.96, reject H_o at α = 0.05;
if the value of Z ≥ 2.58, reject H_o at α = 0.01.

Table 6. Rank sum tabular values at alpha = 0.05 (5) and alpha = 0.01 (1). These values or smaller for the test statistic cause rejection of H_o.

$n_1 \to$	2		3		4		5		6		7		8		9		10		11		12	
$\downarrow n_2$	5	1	5	1	5	1	5	1	5	1	5	1	5	1	5	1	5	1	5	1	5	1
4					10																	
5			6		11		17	15														
6			7		12	10	18	16	26	23												
7			7		13	10	20	17	27	24	36	32										
8	3		8		14	11	21	17	29	25	38	34	49	43								
9	3		8	6	15	11	22	18	31	26	40	35	51	45	63	56						
10	3		9	6	15	12	23	19	32	27	42	37	53	47	65	58	78	71				
11	4		9	6	16	12	24	20	34	28	44	38	55	49	68	61	81	74	96	87		
12	4		10	7	17	13	26	21	35	30	46	40	58	51	71	63	85	76	99	90	115	106
13	4		10	7	18	14	27	22	37	31	48	41	60	53	73	65	88	79	103	93	119	109
14	4		11	7	19	14	28	22	38	32	50	43	63	54	76	67	91	81	106	96	123	112
15	4		11	8	20	15	29	23	40	33	52	44	65	56	79	70	94	84	110	99	127	115

Spearman Rank Correlation Test

This test determines if two variables are related and specifies the degree of relationship. The test statistic is the Spearman rank correlation coefficient, r_s. In perfect positive correlations between two variables, $r_s = +1.0$. In a perfect negative correlation, $r_s = -1.0$. If no relationship exists between two variables, $r_s = 0$. The following procedure is used to obtain the Spearman rank correlation coefficient.

1.   Separately rank each group of variables.

2.   Compute the differences (d) of the ranks for each pair of variables, square these differences (d^2), and sum them (Σd^2).

3.   Use the following formula to obtain r_s.

$$ r_s = 1 - \frac{6 \sum d^2}{n(n^2 - 1)} $$

where: d^2 = square of the difference of the ranks for each pair of variables
          n = number of pairs of variables

4.   The **test statistic** is the calculated r_s for a set of data. Compare r_s with the tabular statistic in Table 7. The tabular statistic equals the smallest allowable r_s in order to assume that a significant correlation exists between two variables (at a specified n and alpha level). Therefore, if the absolute value of the test statistic is equal to or greater than the tabular statistic, reject H_o. If the absolute value of r_s is less than the tabular statistic, you cannot reject H_o.

Example: An investigator wants to determine if a correlation exists between the numbers of cucumber seeds that germinate and the concentration of a specific herbicide to which the seeds are exposed. In the study, cucumber seeds in four dishes (15 per dish) are each wetted with a different concentration of the herbicide. The seeds in a fifth dish, serving as the control, are wetted with distilled water. Therefore, in this study the two variables being tested for a correlation are herbicide concentration and cucumber seed germination. The investigator predicts that a negative correlation should exist between these two variables.

Variable Pair	Herbicide Conc. (mg/l)	Rank	Seeds Germinated	Rank	d	d^2
1	0.000	1	14	4.5	3.5	12.25
2	0.001	2	14	4.5	2.5	6.25
3	0.010	3	12	3	0	0
4	0.100	4	6	2	-2	4
5	1.000	5	2	1	-4	16

n = 5 $\Sigma d^2 = 38.5$

$$r_s = 1 - \frac{6(38.5)}{5(5^2 - 1)}$$

$$r_s = 1 - \frac{231}{120} = 1 - 1.925$$

$$r_s = -0.925$$

H_o: no significant correlation exists between the variables
H_a: a significant correlation exists between the variables
Test Statistic = r_s = -0.925
Tabular Statistic (n=5; α = 0.05) = 0.900

Absolute value of Test Statistic (0.925) > Tabular Statistic (0.900); therefore, reject H_o and assume that a significant correlation (α = 0.05) exists between variables. Negative sign of r_s indicates that it is a negative correlation.

Table 7. Absolute (+ or -) tabular values for the Spearman rank correlation test (n = number of pairs of variables).

	alpha level (α)	
n	**.05**	**.01**
4	1.000	---
5	.900	1.000
6	.829	.943
7	.714	.893
8	.643	.833
9	.600	.783
10	.564	.746
12	.506	.712
14	.456	.645
16	.425	.601
18	.399	.564
20	.377	.534
22	.359	.508
24	.343	.485
26	.329	.465
28	.317	.448
30	.306	.432

*Note: if n > 30 consult Siegel (1956) p. 212.

Chi-Square Test

The chi-square test can be used when (1) the collected data can be classified into exactly one of k categories (i.e., nominal data) and (2) one is interested in determining if observed data frequencies differ significantly from the expected data frequencies predicted by the research hypothesis.

Note: **In this test "frequency" means the number of individuals in a category; it is not a fraction or proportion of all individuals considered.**

In general, H_o states that the observed data frequencies do not differ from those expected and H_a states that they do. The **test statistic**, called chi-square (X^2), is calculated according to the following formula:

$$X^2 = \sum_{i=1}^{k} \frac{(O_i - E_i)^2}{E_i}$$

where: O_i = the observed data frequency in a category

E_i = the expected data frequency in a category

k = total number of categories

In summary, for each 1 to k category one subtracts the expected frequency (E_i) from the observed (O_i), squares the deviation, and divides by E_i. All the squared deviations are added together and the sum equals X^2. Because large differences between observed and expected values will produce large squared deviations, a large X^2 indicates a false H_o. Conversely, a small X^2 indicates a true H_o. The tabular statistic is the **smallest** X^2 value allowable in order to reject H_o at $\alpha = 0.05$. Therefore, if the test statistic is equal to or greater than the tabular statistic, reject H_o at that alpha level. If the test statistic is less than the tabular value, you cannot reject H_o. Table 8 contains the chi-square tabular values indexed according to alpha level and the degrees of freedom (d.f.), where the degrees of freedom equal the number of categories minus one (d.f. = k -1). <u>As with any test, first determine if you can reject H_o at α = .05 (differences are significant), then examine the table to determine if X^2 is large enough to reject H_o at α = 0.01 (differences are highly significant).</u>

The following restrictions must be observed if the chi-square test is to be validly used.

1.   Data being tested must be in frequency form (i.e., number of data items in each category), not in percentage or fraction form.

2.   A chi-square test cannot be used if any expected value is equal to zero, since division by zero is not permissible.

3.   The chi-square test cannot be used if the d.f. equal 1 and any expected value is less than 5.

4.   With d.f. greater than 1, no more than 20% of the expected frequencies can be less than 5.

Example 1: A student captures 23 grass frogs (*Rana pipiens*) in Beebe Lake and hypothesizes that the sex ratio should be 50:50. Upon inspection it is discovered that 9 are males and 14 are females.

i	Categories	Observed (O)	Expected (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
1	males	9	11.5	-2.5	6.25	.544
2	females	14	11.5	2.5	6.25	.544

k = 2 $\qquad\qquad X^2 = 1.088$

H_o: O_i's do not differ signif. from E_i's $\qquad$ H_a: O_i's do differ signif. from E_i's

Test Statistic = $X^2 = 1.088$
Tabular Statistic (α = .05, d.f. = k-1 = 2 - 1 = 1) = 3.84
Test Statistic < Tabular Statistic; therefore, H_o cannot be rejected; differences are not statistically significant.

Example 2: An investigator is studying the inheritance of chlorophyll synthesis in wheat. The following data were obtained in a cross of two genetically different strains of wheat. Each individual was placed into one of three phenotypic classes according to previously defined criteria. The investigator wants to determine if the frequencies of plants of the three phenotypes differ significantly from those expected in a 1:2:1 ratio.

Type	Observed (O)	Expected (E)	(O - E)	$(O - E)^2$	$(O - E)^2/E$
normal	105	120.75	-15.75	248.06	2.05
heterozygous	249	241.50	7.50	56.25	0.23
chlorotic	129	120.75	8.25	68.06	0.56

Total = 483 $\qquad\qquad X^2 = 2.84$

H_o: O_i's do not differ signif. from E_i's $\qquad$ H_a: O_i's do differ signif. from E_i's

Test Statistic = $X^2 = 2.84$
Tabular Statistic (α = 0.05, d.f. = 3 - 1 = 2) = 5.99
Test Statistic < Tabular Statistic; therefore, H_o cannot be rejected; differences are not statistically significant.

Table 8. Chi-square tabular values indexed according to alpha (0.05 to .001) and the degrees of freedom.

Degrees of Freedom	Probability of Obtaining a Value as Large or Larger		
	.05	.01	.001
1	3.841	6.635	10.827
2	5.991	9.210	13.815
3	7.815	11.345	16.268
4	9.488	13.277	18.465
5	11.070	15.086	20.517
6	12.592	16.812	22.457
7	14.067	18.475	24.322
8	15.507	20.090	26.125
9	16.919	21.666	27.877
10	18.307	23.209	29.588

The usefulness of the chi-square test can be extended to situations where several unrelated samples are being compared to determine if they all come from the same population (i.e., if they are or are not significantly different). In the previous examples, data in a single sample (frogs, wheat plants) were classified into two or more discrete categories. Using the following procedure the chi-square test can be used with k samples, each classified into r categories.

1.   Arrange the frequencies in an r x k table.

2.   The null hypothesis states that the r samples of frequencies come from the same population.

3.   Use the following formula in calculating X^2

$$X^2 = \sum_{i=1}^{r} \sum_{j=1}^{k} \frac{[O_{ij} - E_{ij}]^2}{E_{ij}}$$

where: O_{ij} = observed frequency in the ith category from the jth sample
            E_{ij} = frequency in the ith category from the jth sample expected under H_o

4.   The degrees of freedom are calculated according to the formula: d.f. = (r-1)(k-1).

5.   Comparison of test and tabular statistic is as stated previously.

Example 3: An investigator expects that age and sex differences in foraging behavior exist in the black-capped chickadee (*Parus atricapillus*). The following data were collected by following flocks of color-banded chickadees of known age and sex and classifying where birds foraged with respect to the inner, middle, and outer canopy parts of trees. In this analysis the investigator wants to determine if the four samples (age and sex classes) differ with respect to the frequency of foraging sites in the three canopy locations. In the following data table, numbers not in parentheses represent observed frequencies in each class; numbers in parentheses represent expected frequencies based on the H_o that no differences exist in the foraging behavior of the four age and sex classes. The expected frequency of foraging sites for a particular age-sex class and canopy location is calculated by multiplying the sample size for that age and sex class by the fraction of all foraging sites in that canopy location. For example, the expected frequency of adult male foraging sites in the inner canopy equals 74 (total number of adult male foraging sites) times 0.207 (the fraction of all foraging sites in that part of the canopy), or 15.3 foraging sites.

	Canopy Location					
	Inner Canopy		Middle Canopy		Outer Canopy	
Age and Sex Class (r)	O	(E)	O	(E)	O	(E)	Totals
Adult male	21	(*15.3*)	37	(*31.7*)	16	(*27.0*)	74
Juvenile male	26	(*10.6*)	13	(*21.8*)	12	(*18.6*)	51
Adult female	39	(*54.4*)	122	(*112.6*)	102	(*96.0*)	263
Juvenile female	31	(*36.6*)	70	(*75.8*)	76	(*64.6*)	177
	117 = 20.7% of 565		242 = 42.8% of 565		206 = 36.5% of 565)		565

$$x^2 = \sum_{i=1}^{4} \sum_{j=1}^{3} \frac{(O_{ij} - E_{ij})^2}{E_{ij}}$$

X^2 = (21 - 15.3)^2 / 15.3 + (37 - 31.7)^2 / 31.7 + (16 - 27.0)^2 / 27.0 + (26 - 10.6)^2 / 10.6 +
(13 - 21.8)^2 / 21.8 + (12 - 18.6)^2 / 18.6 + (39 - 54.4)^2 / 54.5 + (122 - 112.6)^2 / 112.6 +
(102 - 96.0)^2 / 96.0 + (31 - 36.6)^2 / 36.6 + (70 -75.8)^2 / 75.8 + (76 - 64.6)^2 / 64.6

X^2 = 2.12 + 0.89 + 4.48 + 22.37 + 3.55 + 2.34 + 4.41 + 0.72 + 0.38 + 0.86 + 0.44 + 2.01

X^2 = 44.57

degrees of freedom = (r-1)(k-1) = (3-1)(4-1) = 6

H_o: r_i frequencies for the four age and sex classes do not differ significantly
H_a: r_i frequencies for the four age and sex classes do differ significantly

Test Statistic = X^2 = 44.57
Tabular Statistic (α = .01, d.f. = 6) = 16.8
Test Statistic > Tabular Statistic; therefore, reject H_o and accept H_a at α = .01;
    i.e., differences are highly statistically significant

REFERENCES AND SUGGESTED READINGS

Conover, W.J. Practical nonparametric statistics. New York: John Wiley & Sons. 1980.

Elliot, JM. Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biological Association scientific publication #25. Westmorland: The Ferry House. p. 148. 1971.

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Siegel, S. Nonparametric statistics for the behavioral sciences. New York: McGraw-Hill Co., Inc. 1956.

Snedecor, GW.; Cochran, WG. Statistical methods. 6th ed. Ames, IA: Iowa State University Press. 1989.

Steel, RGD.; Torrie, JH. Principles and procedures of statistics. New York: McGraw-Hill Book Co., Inc. 1960.

White, C. The use of ranks in a test of significance for comparing two treatments. Biometrics 8:33-41. 1952.

Zar, JH. Biostatistical analysis. Englewood Cliffs, NJ: Prentice-Hall, Inc. 1974.

AN EXAMPLE: BIOLOGICAL SCIENCES 1107 SUMMER LABORATORY PRACTICAL

Name: _____   TA's Name: _____

                                                                    Date: _____

I HERBY STATE THAT I WIL NEITHER GIVE NOR RECEIVE INFORMATION DURING THIS EXAM (EXCEPT THAT DELIVERED BY THE LAB INSTRUCTOR), NOR WILL I PASS ON INFORMATION ABOUT THE EXAM TO THOSE STUDENTS WHO HAVE NOT YET TAKEN IT.

                                                            Signed _____

Instructions:

1. Restrict your answers to the questions in the provided space. Answers written in margins will not be graded. Space is provided for calculations, where necessary.

2. Be precise and concise in responding to the questions. Points will be lost for "data dumping!"

3. Good luck!

15 pts **1**. Observe projected slides and/or displays and answer the designated questions regarding each organism. Names of protistan genera and clades observed in lab are provided. Use the abbreviation for the kind of microscopy used.

Genera		**Clades**	**Kind of Microscopy**
Amoeba	*Physarum*	Ameoboza	light (lm)
Euplotes	*Stentor*	Chlorophyta	scanning electron (sem)
Paramecium	*Trichonympha*	Euglenozoa	transmission electron (tem)
Paranema	*Volvox*	Alveolata	
Peridinium	*Euglena*	Radiolaria	
Vorticella	*Actinosphaerium*	Parabasalida	

PROTIST 1.

      A. What is the genus of this organism?   _____

      B. This organism is in which clade?   _____

      C. How does this organism move?   _____

      D. How does this organism obtain food? _____

      E. What kind of microscopy was used to obtain this micrograph? _____

PROTIST 2.

      A. What is the genus of this organism?   _____

      B. This organism is in which clade?   _____

      C. How does this organism move?   _____

      D. How does this organism obtain food? _____

      E. What kind of microscopy was used to obtain this micrograph? _____

<u>PROTIST 3.</u>

      A. What is the genus of this organism? _____

      B. This organism is in which clade?   _____

      C. How does this organism move?   _____

      D. How does this organism obtain food? _____

      E. What kind of microscopy was used to obtain this micrograph? _____

8 pts   **2.** You conduct a series of studies on a newly discovered species of mountain lizard, the push-up lizard (*Arnold schwartzii*), that performs a behavior called "push-ups." The somewhat smaller males seem to perform this behavior slightly more than females. In the first study, you determine the rate of the push-up behavior in lizards held at 14 different environmental temperatures. Your data are shown in Figure 1.
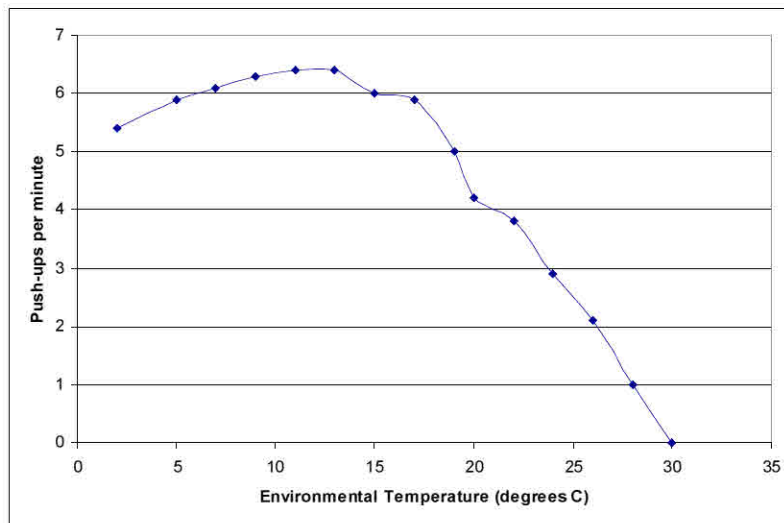
A. What is the dependent variable in this study?

_____

Figure 1. Relationship between environmental temperature (^{o}C) and push-up rate in the push-up lizard (*Arnold schwartzii*)

B. The Spearman rank correlation coefficient (Rs) defining the relationship between these two variables is -.705. Refer to the Spearman rank correlation table of tabular values on page 13 of this exam and complete the following information. Note: the test statistic is the absolute value of the correlation coefficient. The tabular statistic equals the smallest allowable r_s in order to assume that a significant correlation exists between the two variables.

       H$_o$:

       H$_a$:

       Test Statistic =

       Tabular Statistic (n=   ; α=   ) =

       Conclusion

12 pts **3**. Below are the structural diagrams of three nonelectrolytes for which you wish to determine the relative rates of diffusion through the bovine erythrocyte membrane.

```
              H   H   H
              |   |   |
1.    H–O– C– C – C– O–H
              |   |   |
              H   H   H


              H   H   H   H
              |   |   |   |
2.    H–O– C– C – C– C– O–H
              |   |   |   |
              H   H   H   H


              H   H   H   H   H
              |   |   |   |   |
3.    H–O– C– C – C–C– C– O–H
              |   |   |   |   |
              H   H   H   H   H
```
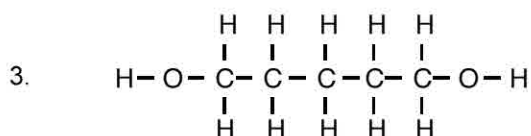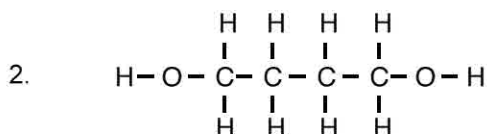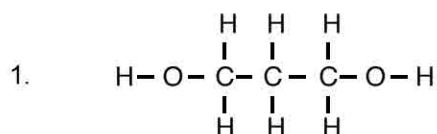
A. Circle on each diagram the portion(s) of the molecule that enables it to interact with and be soluble in a lipid.

B. Which of these nonelectrolytes has the smallest lipid/water partition coefficient?

C. Assume that these molecules can pass through the erythrocyte cell membrane only by simple diffusion (not by facilitated diffusion or active transport). If solutions which are isoosmotic to the cells are used, can you predict in which solution the cells would have the <u>shortest</u> hemolysis time? Explain your answer.

D. Assume that these molecules can pass through the erythrocyte cell membrane only by simple diffusion (not by facilitated diffusion or active transport). The osmolarity of erythrocyte cytoplasm is 0.3 osM. The hemolysis time of erythrocytes placed in a 0.3 osM solution of nonelectrolyte 2 was 200 (+/-10) seconds. If erythrocytes were placed in a 0.2 osM solution of nonelectrolyte 2, would their hemolysis time be **longer than, equal to** or **shorter than** 200 (+/-10) seconds? Explain your answer.

4 pts   **4**. Use the compound microscope to answer this question. Please do not change the objectives on the microscope.

Use the mechanical stage and vernier to measure the diameter of the root cross-section on the slide. Make sure to include appropriate units.

Initial reading: _____

Final reading: _____

Diameter: _____

8 pts   **5**. The movement of sap (water and solutes) through the xylem tubes from the roots to the leaves of small plants may be accomplished by root pressure (a positive pressure) or a negative pressure (tension) on the sap. In the blank beside each mechanism below, list the numbers of all the conditions listed below which would increase the contribution of that mechanism for the movement of sap in the xylem tubes. (Note: one or more of the conditions may not apply to either mechanism.)

A. Root pressure mechanism: _____

B. Transpiration-cohesion-tension mechanism: _____

Conditions
1. Leaf stomata open
2. Leaf stomata closed
3. No air movement over the plant
4. 30 km/hr air movement over the plant
5. Plant roots in a soil deficient in all
   mineral nutrients
6. Plant roots in a soil with sufficient
   mineral nutrients

7. Plant in air with 20% relative humidity
8. Plant in air with 100% relative humidity
9. Plant roots in very dry soil
10. Plant roots in water-saturated soil
11. Plant in the dark
12. Plant illuminated with a 100-watt lamp

5 pts   **6**. Use the microscope on the TA's desk to answer this question.

Note: you **have five minutes to complete this question**. When you are finished, indicate to your TA that your work is ready to be graded.

Two cross sections of a *Ligustrum* leaf are mounted on the slide on the microscope.

A. Focus on one of the sections, finally using the 40X objective lens.

B. Position the section so that the tip of the point in the ocular indicates a **stoma**.

6 pts   7. Referring to specific bones and muscle attachment points on the diagrams below, give **one** feature of the mole's front limb that is an adaptation for digging and explain why it is an adaptation for that activity.

B

radius

ulna

front
foot
without
details

humerus

scapula

elongated
"wrist" bone

clavicle

sternum

C

radius

clavicle

ulna

humerus

keel of
sternum
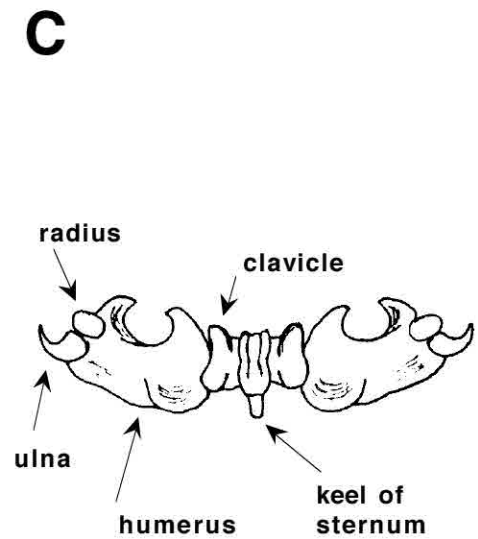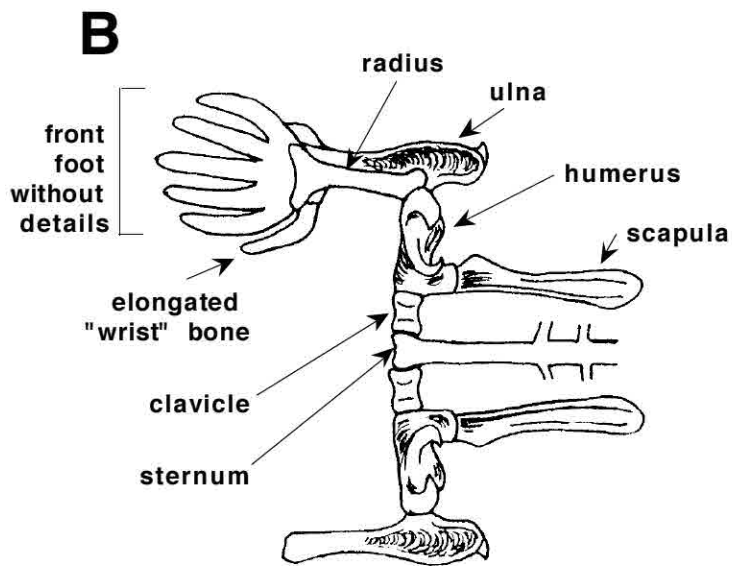
Below is a diagram of a cross section of a vascular bundle.
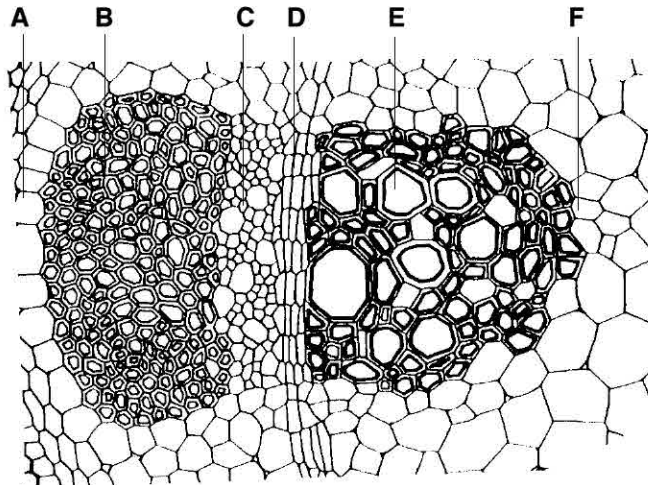
1 pt   **8**. Identify the type of vascular tissue indicated by line C. _____

1 pt   **9**. Give the letter of the line that indicates the vascular cambium. _____

1 pt   **10**. In which plant organ is this vascular bundle? (Circle correct answer.)

        1.leaf   2.root   3.stem

4 pts   **11**. The foramen ovale is a structure found between the right and left atria of the fetal pig heart that allows shunting of blood to bypass the lungs. Name and give the location of two other circulatory structures present in a fetal pig but absent in an adult animal.

10 pts   **12**. When you stand on your toes with your heel lifted off the floor, you are using your foot as a second class lever. The fulcrum of the lever is at the ball of the foot (indicated by the triangle in the diagram below). Contraction of the calf muscles causes the Achilles' tendon to lift the heel and also lift the weight of the leg and everything supported by the leg. The position of this weight (the out force) on the foot is indicated by the arrow. Note that this is a different class of lever than observed by the model in lab.

A.   On the diagram below <u>draw and clearly label</u> the in- and out- moment arms, then measure the lengths in millimeters of those moment arms.

S_i = _____ S_o = _____

B.   Suppose you weigh 60 N and you are standing on one foot with your heel raised (as in the diagram below). How much force is produced by the contraction of the calf muscles (the in-force)? Show your work.

F_i = _____

C.   In general, during the evolution of a population of animals, what parts of a lever system may change to increase the speed ratio? Circle all that apply.

S_i longer   **S_i shorter**

S_o longer   **S_o shorter**

Achilles
tendon

Ligaments
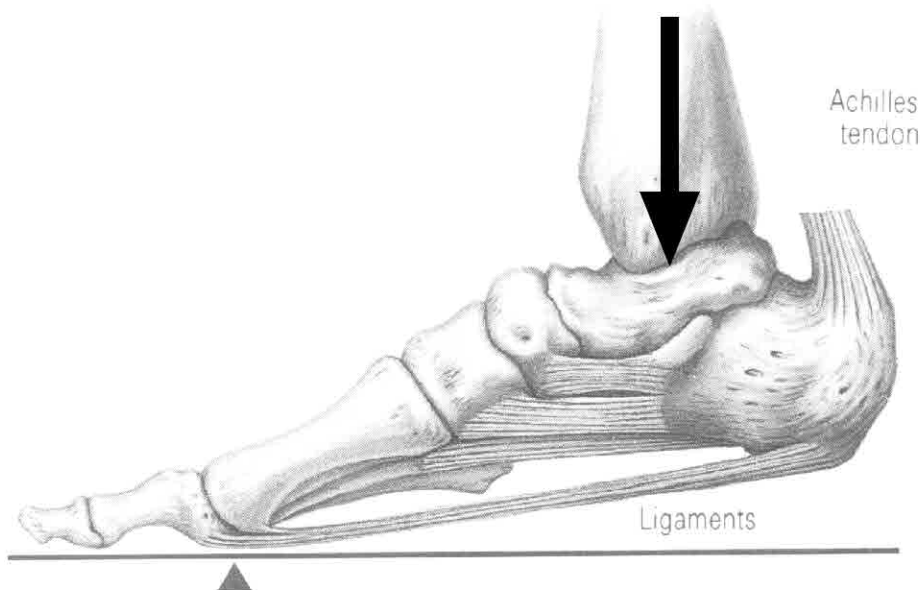
13 pts **13.** The drug 2'3' dideoxyinosine (ddI) has potent activity against human immunodeficiency virus (HIV). Unfortunately, the deactivation of ddI is catalyzed by the human plasma enzyme purine nucleoside phosphorylase (PNP). You are part of a team to design an inhibitor for PNP that can be used in conjunction with ddI to render it more effective against HIV. Your group has synthesized three potential PNP inhibitors (x, y, and z) and it is your job to test their effectiveness. You set-up four velocity vs. substrate concentration studies; in A, PNP is tested by itself, in B, C, and D, PNP is tested with one of the drugs you designed. In all studies, the PNP concentration is the same. Figure 2 shows your results.
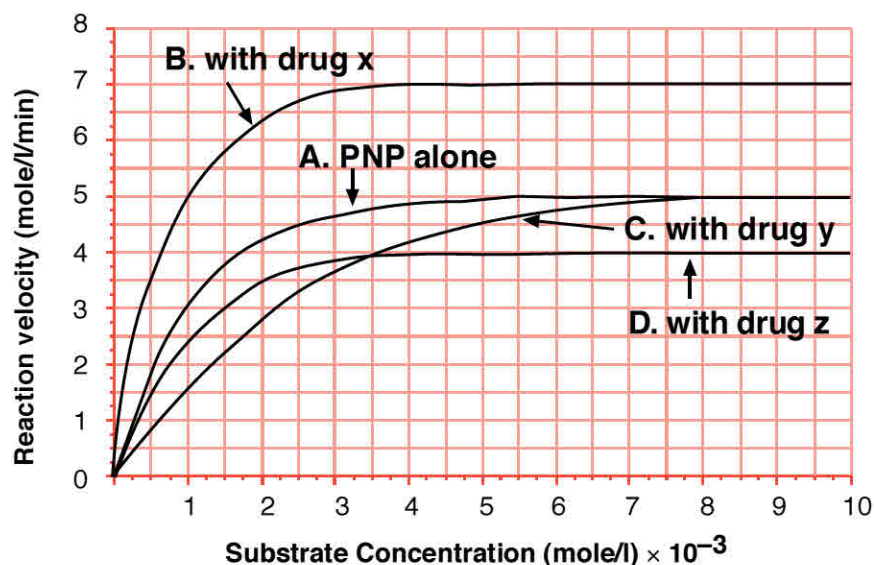
Figure 2. Velocity vs. substrate conc. curve for the enzyme purine nucleoside phosphorylase (PNP) by itself (A) and with drug x (B), y (C), and z (D).

A. What is the substrate in this study? _____

B. Estimate V_m and K_m from each curve and enter these values in Table 1.

Table 1. Estimates of V_m and K_m from curves A, B, C, and D shown in Figure 1.

CURVE	K_m	V_m
A (PNP alone)		
B (with drug x)		
C (with drug y)		
D (with drug z)		

C. Based on the data presented in Table 1, discuss the specific effect that **drug y** has on the enzyme PNP. If you think that **y** is an inhibitor, indicate its type, and discuss your reasoning. What is this drug's potential to be used in conjunction with ddl as an HIV therapy?

D. Based on the data presented in Table 1, discuss the specific effect that **drug z** has on the enzyme PNP. If you think that **z** is an inhibitor, indicate its type, and discuss your reasoning. What is this drug's potential to be used in conjunction with ddl as an HIV therapy?

off the mark.com   by Mark Parisi

PARAMECIUM PRANKS

© Mark Parisi, Permission required for use.
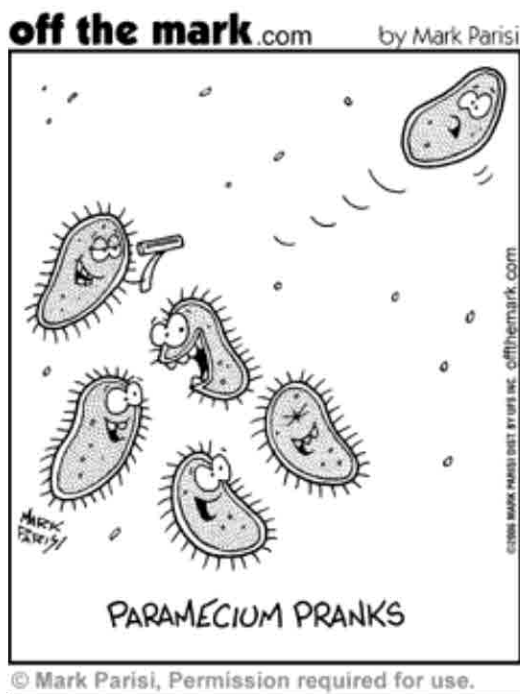
12 pts **14.** Many algae possess a form of the enzyme chlorophyllase which is not denatured by a high concentration of acetone; consequently, this enzyme can function during the acetone extraction of chloroplast pigments. Chlorophyllase catalyzes the hydrolysis of chlorophyll a which produces phytol and chlorophyllide a, shown in the diagram below.

chlorophyll a

chlorophyllase H_2O

phytol   **chlorophyllide a**
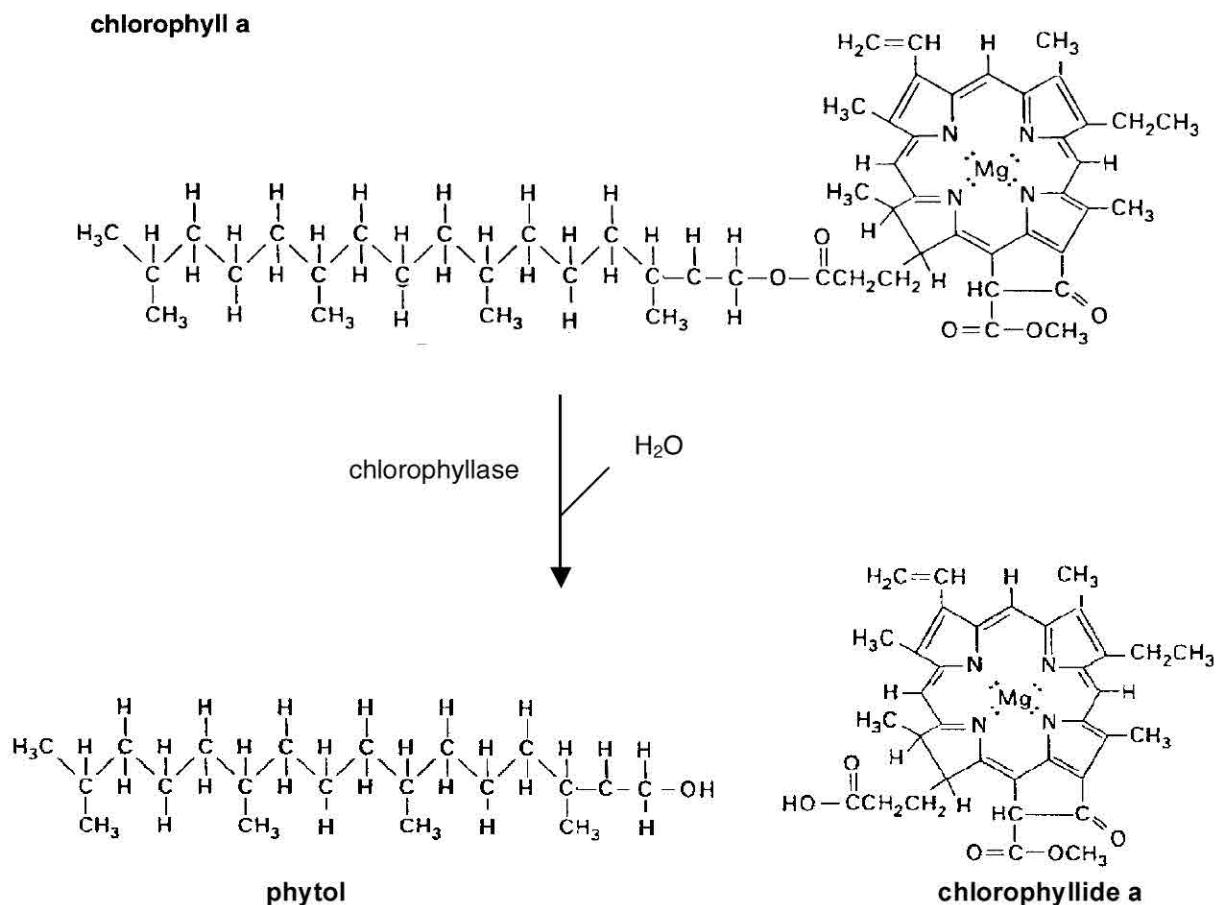
Both phytol and chlorophyllide a are soluble in acetone and petroleum ether, so they could have been present in one or more of the pigment mixtures your lab section prepared for chromatography. Suppose phytol and chlorophyllide a were present in a mixture of chloroplast pigments that were separated using the thin layer chromatography system we used in the laboratory.

A. Where on the chromatogram would the **phytol** be relative to the chlorophyll a? (Circle your answer)

Closer to the origin   **Further from the origin**

Explain your answer to part A.

B. Where would the **chlorophyllide a** be relative to the chlorophyll a? (Circle your answer)

Closer to the origin   **Further from the origin**

Explain your answer to part C.

7 pts   **15**. Examine the dissected fetal pig associated with this question.

A. Is the individual shown here a male or a female? Give two observable features that helped you arrive at your decision.

B. Identify **3 of the 5** structures indicated by the numbered pins.

**STRUCTURE**

1   _____

2   _____

3   _____

4   _____

5   _____

7 pts   16. A.   Match the number of the indicated cell, part, or region on the leaf section diagram to its name on the right of the diagram.
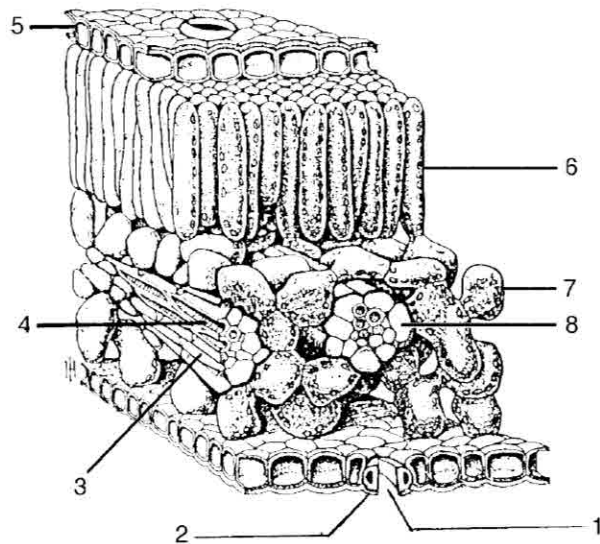
_____ bundle sheath cell

_____ epidermis

_____ palisade mesophyll

_____ phloem

_____ spongy mesophyll

_____ xylem

B.   Give the number of the cell type composing the tissue from which the _greatest_ amount of water evaporates.

_____

5 pts   17. Below is a diagram of the ventral side of a fetal pig heart. Please write the names of the indicated structures.
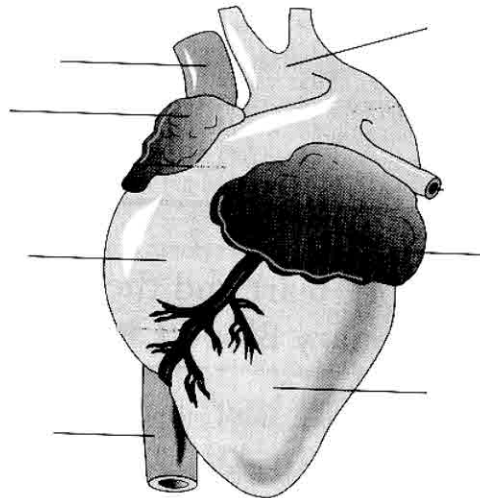
A.   The fetal pig heart has four chambers. Which one would have blood with the highest O_2 concentration?

_____

B.   What vessel brings blood to the heart from the placenta? _____

Table 1. Absolute (+ or -) tabular values for the Spearman rank correlation test (n = number of pairs of variables).

n	Alpha level (α)	
	0.05	0.01
4	1.000	---
5	0.900	1.000
6	0.829	0.943
7	0.714	0.893
8	0.643	0.833
9	0.600	0.783
10	0.564	0.746
12	0.506	0.712
14	0.456	0.645
16	0.425	0.601
18	0.399	0.564
20	0.377	0.534
22	0.359	0.508
24	0.343	0.485
26	0.329	0.465
28	0.317	0.448
30	0.306	0.432

THE END!! BE SURE YOU HAVE ANSWERED ALL THE QUESTIONS.

HAVE A GREAT DAY AND FEEL GOOD ABOUT YOUR ACCOMPLISHMENT!!!!!

BioG 107 Summer 2007 Lab Practical Answer Key

1. (1 point each) Protist 1
   A. Trichonympha
   B. Parabasalida
   C. multiple flagella
   D. phagocytosis
   E. sem
   Protist 2
   A. Paramecium
   B. Alveolata
   C. numerous cilia
   D. ingestion at oral groove
   E. lm
   Protist 3
   A. Actinosphaerium
   B. Radiolaria
   C. drifts with water currents
   D. phagocytosis after food item sticks to axopodia
   E. lm

2. A (1 pt). push-ups/minute
   B (1 pt each). H_o: There is no significant correlation between environmental temperature and push-ups/minute in the push-up lizard
   H_a: There is a significant correlation between environmental temperature and push-ups/minute in the push-up lizard
   (1 pt) Test Statistic = 0.705
   (1 pt) Tabular Statistic: (n=14; α=0.01) = 0.645   or   (n=14; α =0.05) =0.456
   (3 pts) Conclusion: Reject H_o; There is a significant negative correlation between environmental temperature and push-ups/minute in the push-up lizard

3. A (3 pts). hydrocarbon groups should be circled (-OH groups should not be circled)
   B (1pt). 1
   C. (2 pts for yes/no, 2 pts for reasoning) No. In general, the ability of a molecule to pass through the membrane increases with its lipid solubility and decreases with its molecular size. In the case of these 3 molecules, their lipid solubility increases with their size, so the two characteristics may counteract in determining the diffusion rates of the molecules through the membrane.
   D. (2 pts for answer, 2 pts for reasoning) Shorter than. The 0.2 osM solution has a greater Ψ than the 0.3 osM erythrocytes. Therefore, when the erythrocyte is placed in the 0.2 solution, water will immediately enter the cell resulting in a shorter hemolysis time.

4. (4 pts) diameters in the range of 1.0 –1.5 mm.

5. (8 pts)
   A. 2, 3, 6, 8, 10, 11
   B. 1, 4, 5, 7, 9, 12

6. (5 pts) microscope question

7. (6 pts; 3 pts for example, 3 points for explanation)
   Digging requires force and not speed. Adaptations you could have mentioned include:
   - specific muscle attachment points and their arrangements
   - the unusually long in-distance provided by the extension of the ulna (elbow)
   - the restricted rotation of the humerus; its only possible motion is that of digging
   - the thickness of the humerus, to handle the forces it transmits
   - the shortened radius, for reduced out-distance

8. (1 pt) phloem

9. (1 pt) D

10. (1 pt) stem

11. (2 pts) ductus arteriosis – connection between pulmonary aorta and systemic aorta
    (2 pts) Ductus venosus – joining of umbilical vein and hepatic portal vein

12. A. S_i and S_o are the perpendicular distances from the triangle (the fulcrum) to the lines of force on the Achilles' tendon and from the weight of the leg on the foot, respectively. The most accurate way to measure S_i and S_o is to draw lines perpendicular to the horizontal line under the foot from:
    1. the midpoint of the attachment site of the tendon (this is F_i's line of force)
    2. the tip of the arrow on the leg bone (this if F_o's line of force)
       S_i is the horizontal distance from the triangle to F_i's line of force. $S_i = 81$ mm
       S_o is the horizontal distance from the triangle to F_o's line of force. $S_o = 58$ mm
       (4 pts; 2 pts for labeling diagram, 2pts for measurements)
    B. (4 pts) $F_o S_o = F_i S_i$
       $(58)(60) = F_i (81)$
       $F_i = 42.96$ N

    C. (2 pts) S_o longer and S_i shorter

13. A. (1 pt) ddI
    B. (5 pts: 1/2 pt each + 1 pt for correct units)

CURVE	K_m	V_m
A (PNP alone)	0.75×10^{-3} mol/l	5.0 mole/l/min
B (with drug x)	0.5×10^{-3} mol/l	7.0 mole/l/min
C (with drug y)	1.75×10^{-3} mol/l	5.0 mole/l/min
D (with drug z)	0.75×10^{-3} mol/l	4.0 mole/l/min

(C, D: 4 pts each: 1 for specific effect, 1 for type of inhibitor, 1 for reasoning, 1 for therapy potential)
C. The effect of drug Y can be overcome by adding more substrate. Drug Y is a competitive inhibitor. It increases the K_m, but V_m stays the same. This would not be an effective drug for HIV therapy, because ddI would still be deactivated at the same rate.

D. Drug Z reduces the number of effective enzyme molecules in the system. Drug Z is a noncompetitive inhibitor. It decreases the V_m, but K_m stays the same. This drug would be good for HIV therapy because it would result in ddI being deactivated at a decreased rate.

14. A. (2 pts) further from the origin.
    (4 pts) Phytol is less polar than chlorophyll a and would be more attracted to the mobile phase, less attracted to the stationary phase, and will move further with the mobile phase
    B. (2 pts) closer to the origin.
    (4 pts) Chlorophyllide a is more polar than chlorophyll a, would be more attracted to the stationary phase, less attracted to the mobile phase, and won't move as far with the mobile phase

15. A. (3 pts) Female: genital papillae, uterine horn, ovaries
    B. (3 pts)
        1. lung
        2. trachea
        3. bladder
        4. pancreas (in Kathryn Bushley's lab this was the gall bladder)
        5. spleen

16. (1 pt each answer)
    A. bundle sheath cells: 8
       Epidermis: 5
       Palisade mesophyll: 6
       Phloem: 3
       Spongy mesophyll: 7
       Xylem: 4
    B. 7

17. (1/2 pt each answer)
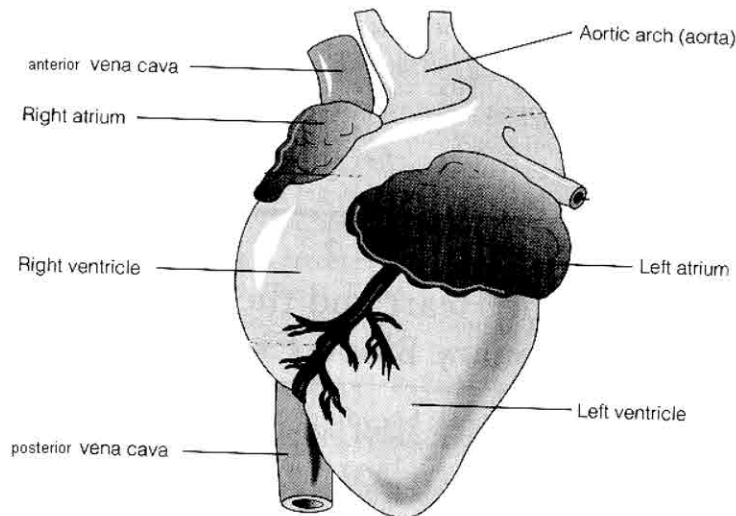
    A. Right atrium
    B. Posterior vena cava

BIO 1108   **Course Information and Policies**   Scott T. Meissner, Lecturer
2010
Welcome to summer Introductory Biology!

The following deals mainly with items relating to the lecture and to general course policies.
Please see the course calendar and the Laboratory Information handout for further information on
the laboratory part of the course.

Course staff and contact information: E-mail:
    Visiting Lecturer: Scott T. Meissner   stm4@cornell.edu
    Lab Coordinator: Mark Sarvary   mas245@cornell.edu
    Teaching Assistants: Luis Duque   lod3@cornell.edu
        Corey Ptak   cjp57@cornell.edu
    Course Coordinator: Louise Lattin   lsg6@cornell.edu
The course administrative office is 1140 Comstock Hall. The telephone number is 255-2031.

Texts:

    Our text is *Biology*, 8th edition, 2008, by Campbell et al. New and used copies are
available at the campus bookstore. A copy is available for use in room 1122 Comstock Hall.
    The BIO 1108 laboratory manual will be handed out in class.

Lectures and Labs:

    Lectures will be held from 9:00-12:00 am on Mon., Tues., Thurs., Fridays in room A106
of Corson-Mudd Hall. At the end of this handout are given a calendar of lecture topics and a list
of reading assignments for each lecture.
    Labs will meet from 1:30-4:30 pm Mondays through Thursdays. Each lab will start with
an organizational meeting in room 1116 Comstock Hall. Please see the laboratory general
information handout for more details on the lab part of this course.

Course website:

    The course website is: http://blackboard.cornell.edu/. To log on to this site please use
your Cornell Net ID as your username, and use your CU NET password. Once logged in you
should click on the BIO 107-108 link. If you are visiting from another campus, and do not have a
Cornell Net ID, you should visit the Computer Center and get one, and the sign up for a
blackboard account. If you have problems logging in please let me know.
    In the website you will find links to answer keys, links to campus policies, copies of some
handouts, etc.... Copies of my lecture notes will be posted, usually within a day after each lecture.
Many documents will be posted as PDF files, which can be read by Acrobat reader.
    Please check your e-mail regularly in case you are sent course announcements.

Office hours:

You may come to my office hours to do several things: Ask me questions about course material, describe items to me to get feedback on your mastery of a topic, or just bring a bag lunch and sit in a soft seat before going to lab in the afternoon... No appointment needed.

I will hold office hours at the following times in room 1122 Comstock Hall:
      12:25-1:25,   Monday through Friday, and also
      10:00-12:00,   Wednesday mornings.

Other times may be possible by appointment, especially earlier Wednesday mornings or later Friday afternoons. Questions sent by e-mail will be joyfully received and answered when I can. I am happy to discuss items from lecture, or lab, as you see fit.

Points and assigning letter grades:

You will receive one letter grade for BIO 1108, which will include points from lecture and from lab. The letter grades for BIO 1108 will be set relative to the highest number of points **actually earned** by someone in the course. As a rough guideline: Anyone who earns 90-100% of that highest earned score will be in the "A" range (A^+, A, A^-), 80-90% of the highest score will earn a grade in the "B" range (B^+, B, B^-), 70-80% of the highest score will be the "C" range, 60-70% of the highest score will be the "D" range, and below 60% of the highest earned score will be failing. I reserve the option of considering such additional issues as attendance, improvement in the course, participation, and other factors when determining course grades.

There will be 300 total points for lecture and 300 total points for lab. For a total of 600 points. Here is the breakdown of the points that will be assigned for BIO 1108:

Lecture	Exam 1	70 points
	Exam 2	70 points
	Exam 3	145 points
	Three Homework Sets (5 points each)	15 points
Laboratory	Quiz 1	42 points
	Quiz 2	42 points
	Cell Division Worksheet	15 points
	Sordaria Worksheet	15 points
	Biochemical Pathways Worksheet	16 points
	Restriction Mapping Worksheet	16 points
	Plant Life Cycles Worksheet	16 points
	Angioperm Growth Worksheet	15 points
	Freshwater Ecology Writing Assignment	23 points
	Practical Exam	100 points
	Total for entire course:	600 points total

Exams:

There will be three exams for BIO 1108. Lecture exams will cover material from lecture only, not from the lab. The following is the coverage for each exam:

Exam 1: 9:00-11:00 am, July 20; covers material from lectures #1-12.
Exam 2: 9:00-11:00 am, July 29; covers material from lectures #13-24.
Exam 3: 9:00-noon, August 9, covers material from all lectures.

Homework sets:

The purpose of these homework sets is to give you practice with some of the material before confronting it on exams. Be aware that since these homework sets are "open book" some of the questions in them go into more detail than I would expect of you on exams. The intent is for you to get feedback on the material before each lecture exam, but since it may take me a while to grade these sets, I suggest that you keep a photocopy or scan of your work so that you can immediately compare your answers to the posted answers before each exam.

Homework set 1:	Covers lectures 1-12.	Due: Monday July 19.
Homework set 2:	Covers lectures 13-24.	Due: Tuesday July 27.
Homework set 3:	Covers lectures 25-33.	Due: Tuesday August 3.

Each of these homework sets will be due at or before the start of lecture (9:00 am) on their due date. Homework sets handed in late will suffer a loss of two points out of the five each homework set is worth. No homework set will be accepted for grade if turned in after its answer key is posted (typically by noon of the day it is due).

Academic integrity and acknowledging the work of others:

As pre-professionals you are encouraged to discuss the material covered in this course with one another. However, the answers you give on exams, homework sets, or other assignments in this course are expected to be your own work, given in your own words.

For the sake of homework sets it is assumed that the source of facts used in answers is either the textbook or the lecturer, and for such cases no citing of a source of facts is needed. But in cases where you choose to make use of the writing of others (whether actual words or the flow of ideas, or to make use of an apt phrase, to paraphrase, or to quote from any source), you are expected to clearly indicate what part(s) of an answer are your writing and what part(s) are from another source. In such cases you are required to both cite the source appropriately and use quotation marks as needed. Failure to do so may result in a loss of points on an assignment, or in extreme or repeated cases lead to a charge of academic misconduct.

The Writing Appendix of the lab manual has information concerning how to appropriately cite sources, and information about relevant policies of Cornell can be found in the publication The Code of Academic Integrity and Acknowledging the Work of Others (available in the course web site and at: http://web.cornell.edu/UniversityFaculty/docs/AI.Acknow.pdf).

Answer keys and requests for regrades:

Suggested exam and homework answers will be posted in the hallway leading to 1122 Comstock, and on the course website, after each exam and due date. In many cases questions can have more than one correct answer, and for many questions partial credit may be earned even for incorrect answers. You may (indeed I hope you will!) wish to discuss the questions and answers with me after taking exams.

If you wish to request that the grading of a question used in lecture be reconsidered please note the following: I suggest that you first check the posted answer keys, the text, and then come and talk with me about any items before you request a formal regrade of an item. For most lecture items (except for the final exam) you will have until noon on the last day of the course (August 9) to submit written arguments requesting regrades. Each request should be accompanied by the original graded item and a description of your reasons for reconsideration. For exam #3 you will have until noon of Tuesday, August 10, to e-mail me comments about possible alternate answers. I will try to be generous when reconsidering items, but you can help by presenting me with tightly reasoned arguments pointing out either an error in grading, or an alternate answer for a question, and by supporting your arguments where possible by references to our text or other sources.

Please see the lab coordinator concerning regrading of any lab-related materials.

Absences and last day to withdraw:

Attendance at lecture and laboratory is required. This is an accelerated course with material being covered very rapidly. Thus each day of lecture and laboratory in this course is equivalent to a week's worth of material from a course in the regular semester. Be aware that no make-up exams are planned. If you fall ill, or if something prevents you from doing the work for this course please notify me as soon as possible.

The last day you can withdraw and arrange to have a full refund of tuition is the third day of the course (July 14).

How I suggest you approach this course:

Each day of lecture will usually run for three hours. Each hour of lecture has reading assignments which often comprise a chapter of the textbook. And then there is the lab material. You should expect to spend evenings and weekends focusing on the material from this class. Here is some advice for approaching the lecture material of this course.

For each lecture I will give you a set of learning objectives. My goal is for the majority of the questions on the exams to have some connection to those objectives. But there may be some questions that may not be explicitly stated in the objectives.

I view the text book readings as mainly supplementary and as another means for you to get information on critical items raised in lecture. Use the book mainly as a means to expand on the concepts raised in lecture, to review the definitions of key terms, or to review important structures and functions, as well as to explore new examples and implications of those concepts. In some of the lectures' learning objectives I have noted questions at the end of chapters in the text that I feel might be useful to you, be sure to try them and see the appendix in the back of the text for the answers. Use the learning objectives and the items covered in lecture as a guide to focus your attention as you read. This means that you should indeed read the assigned reading, but do not try to learn everything in the text. Rather you should attempt to master all the items raised in each lecture's learning objectives, and be able to consider implications of that material.

Here is what I suggest that you do before coming to each lecture: Review the learning objectives for that lecture and get a sense of what sort of topics you will be expected to master. You might want to jot down a list of topics or concepts that you identify as key ones, just to fix them in your mind a bit better. Then skim through the assigned readings and pay particular attention to areas that you can identify that relate to the lecture's objectives. Each chapter of the text has a good summary section at its end and some people find this helpful.

Then come prepared to listen and take notes at lecture. This means being awake and taking good active notes. Don't fall into the trap of trying to write down each and every word, but do attempt to synthesize and summarize key points as you identify them.

After lecture: Review your notes and read the text more deeply to get another point of view on the material. My posted lecture notes include a list of all the figures from the text that I use in each lecture. So you should be able to use them to help you focus in on the parts of the text that are most relevant. You should run through the learning objectives several times, daily is ideal, and quiz yourself on important terms, concepts, structures and processes. Identify topics that may need more attention and spend some time working on them. Review those specific topics by reviewing sections of the text, or by referring to my posted lecture notes. Starting to work on the question(s) in the homework set that relate to that day's lectures is another good thing to do.

As you do this cycle of studying and reviewing you may come up with questions that you may wish to ask me. Please do contact me as you feel the need. If you have a burning issue at midnight you can send me an e-mail, but be aware that I will most likely be asleep and will not read it until the next morning. But I will most likely be able to send you a reply before lecture. Another approach is for you to note your question and bring it to my office hours the next day. In the past I have been happy to have many people who took this course use both of these

approaches, and there have been many students with whom I had multiple contacts each week.

Most importantly, keep plugging away at the material, and stay in touch with me and the laboratory staff via e-mail or at office hours. In the past some good hard work has seen many students through this course and on to a good outcome, and hopefully this will be the case for you as well!

I look forward to working with you!   STM

Reading assignments for BIO 1108 Lectures:

Date:	Lecture #	Topics:	Readings: (Campbell et al., 2008)
July 12	1)	Eukaryotic Life Cycles	Pgs. 248-253, 638-640, 812-813, 997-1000, and figures 13.5, 13.6, 28.11, 28.22, 29.5 (Alternation of Generations), 29.13, 31.5, 31.19
	2)	Cell Division	Chapter 12.
	3)	Mendelian Genetics and Meiosis	Chap. 13, and pgs. 262-271.
July 13	4)	Post-Mendelian Genetics	Pgs. 271-281.
	5)	Chromosome Structure	Chapter 15.
	6)	Fungal Diversity	Chapter 31.
July 15	7)	DNA as Genetic Material	Pgs. 86-89, 305-310.
	8)	DNA replication	Pgs. 311-319.
	9)	Transcription and RNA Processing	Pgs. 320-322, 325-336, 347, 356-358, 362-366.
July 16	10)	Translation	Pgs. 328-331, 337-344, 346-348.
	11)	Control of Gene Expression I	Pgs. 346-347, 351-358, 362-366.
	12)	Gene Expression II	Pgs. 300-301, 320-323, 356-366, 432-442, 939-940.
July 19	13)	Viruses	Chapter 19, and fig. 6.2
	14)	Prokaryotic Diversity	Pgs 551-553 and Chapter 27
July 20	15)	Recombinant DNA I	Chapter 20.
July 22	16)	Fertilization	Pgs. 206-207, 806-807, 812-815, 997-1003, 1021-1025, and figures 46.12 and 46.15.
	17)	Angiosperm Life Cycle	Pgs. 252-253, 600-606, 618-621, 625-634, 801-811.
	18)	Plant Kingdom and Evolution	Chapter 29, and pgs. 618-625.

Date:	Lecture #	Topics:	Readings: (Campbell et al., 2008)
July 23	19)	Immune System	Chapter 43.
	20)	Animal Development I	Pgs. 1025-1044.
	21)	Animal Development II	Pgs. 223-225, 366-373, 412-416, 442-447, 757-761
July 26	22)	Phylogeny and Systematics	Chapter 26.
	23)	Mutations	Pgs. 297-302, 316-318, 344-346, 356-363, 373-377, 432-442, 495-496, and 576-577.
	24)	Recombinant DNA II	Pgs. 561-564, and chapter 20.
July 27	25)	Darwinian Evolution	Chapter 22.
	26)	Invertebrate Diversity	Chapters 32 and 33.
July 29	27)	Bioassays	Pgs. 583-584 (malaria), 798 (dodder), 821-841, and Lacroix et al. (2005), and Runyon et al. (2006).
July 30	28)	Post-Darwinian Evolution	Chapter 24, and pgs. 483-484, 525-531.
	29)	Population Genetics	Chapter 23.
	30)	Pollination Ecology	Pgs. 630-632, 804-805, 1198-1203.
Aug 2	31)	Origin(s) of Life	Pgs. 58-59, 507-525.
	32)	Ecology, abiotic factors	Chapter 52, and pgs. 1226-1228.
	33)	Population Ecology	Chapter 53.
Aug 3	34)	Community Ecology	Chapter 54.
	35)	Ecosystems	Chapter 55.
	36)	Conservation Ecology	Pgs. 1236-1242, and chapter 56.

BIOLOGICAL SCIENCES 1108 [12 JULY - 9 AUGUST, 2010]

	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
Lectures Week 1	12 July 1) Intro, Life Cycles 2) Cell Division 3) Mendel	13 July 4) Post-Mendelian Genetics 5) Chromosomes 6) Fungal Diversity	14 July Office Hours	15 July 7) DNA as genes 8) DNA replication 9) Transcription	16 July 10) Translation 11) Gene expression I 12) Gene expression II
Laboratory Week 1	Cell Division; Biochemical Pathways I	*Sordaria* Genetics I	*Sordaria* Genetics II **(Cell Div. Wkst Due)**	Biochemical Pathways II **(*Sordaria* Wkst Due)**	
Lectures Week 2	19 July **(Hwk Set #1 Due)** 13) Viruses 14) Prokaryotic Diversity (review)	20 July **Exam #1** 15) Recombinant DNA I	21 July Office Hours	22 July 16) Fertilization 17) Angiosperm Life Cycle 18) Plant Kingdom	23 July 19) Immune system 20) Animal Development I 21) Animal Development II
Laboratory Week 2	Recombinant DNA I **(Biochem. Path. Wkst Due)**	Recombinant DNA II Freshwater Ecology	Sea Urchin Fertilization Freshwater Ecology	Plant Diversity **Quiz #1**	
Lectures Week 3	26 July 22) Phylogeny and Systematics 23) Mutations 24) Recombinant DNA II	27 July **(Hwk Set #2 Due)** 25) Darwinian Evolution 26) Invertebrates (review)	28 July Office Hours	29 July **Exam #2** 27) Bioassays	30 July 28) Post-Darwinian Evolution 29) Population Genetics 30) Pollination Ecology
Laboratory Week 3	Recombinant DNA III Angiosperm Growth and Development I **(Plant Life Cycle Wkst Due)**	Invertebrates I	Invertebrates II **Quiz #2** **(Rest. Mapping Wkst Due)**	Freshwater Ecology	
Lectures Week 4	2 August 31) Origin(s) of life 32) Ecology, abiotic factors 33) Population Ecology	3 August **(Hwk Set #3 Due)** 34) Community Ecology 35) Ecosystems 36) Conservation Ecology	4 August Office Hours	5 August Office Hours	6 August Office Hours
Laboratory Week 4	Angiosperm Growth and Development II	Field Ecology	Lab Review **(Angiosperm Growth Wkst Due)** **(Fresh. Ecol. Writing Assign. Due)**	**Lab Practical Exam**	
Lectures Week 5	9 August **Final Exam**				

BIO 108   2010
Day 1, Lecture 1, Title: Eukaryotic Life Cycles.
Text Readings: Campbell et al. (2008), pgs. 248-253, 638-640, 812-813, 997-1000, and figures 13.5, 13.6, 28.11, 28.22, 29.5 (Alternation of Generations), 29.13, 31.5, 31.19.

Objectives:

What is the difference between haploid and diploid? What other types of ploidy might be possible in eukaryotes, and what types are unlikely to occur in sexual life cycles? What processes are used by eukaryotes to alter their ploidy during their life cycles? Be able to consider the advantages and disadvantages of sexual versus asexual life cycles.

Be able to distinguish and describe the differences between the life cycles of a typical: animal, plant, fungus, and single-celled green alga. In each case what process produces a zygote? In each case what process produces a gamete? In each what is formed as a result of meiosis? Be able to identify any "generation" in the life cycle as well as the presence of any spores and/or dikaryotic stage.

Fertilization (i.e. syngamy) is made up of stages called plasmogamy and karyogamy. What happens in each of these stages and how do these two stages differ?

Which stages of a fungal life cycle can be considered to be haploid, diploid, or dikaryotic? How is a ciliate protist, before it has completed fertilization, similar to that of a dikaryotic fungal cell?

Note: When you look at figures 28.22, 29.13 and 31.19 do NOT worry about the detailed names of specific structures given in these figures. Do worry about being able to locate in a similar figure such items as: Meiosis, mitosis, gametes, any spores, the zygote, and the stages of fertilization. Also be able to describe the ploidy (1N, 2N, and any N+N) of the various stages in these life cycles.

BIO 108   2010
Day 1, Lecture 2, Title: Cell Division.
Text Readings: Campbell et al. (2008), chapter 12.

Objectives:

Be able to describe how cyclin and cyclin-dependent kinases are used to control the transition between various stages of the cell cycle.

Know the stages of mitosis (and meiosis, which will be covered in a subsequent lecture). Fig. 12.6 in the text is a useful summary of events, but be able to generalize it to other species that have a different number of types of chromosomes than are shown in this figure. What event(s) are associated with the start and completion of each stage?

Both mitosis and meiosis involve the process of segregation. During mitosis what is being segregated and why is this critical? Mitosis is said to be a conservative type of cell division while meiosis is a reductive type of cell division; what is being conserved and what is being reduced in each of these cases?

How do chromosomes at the start of cell division differ from chromosomes at the end of cell division? What are homologous chromosomes, what makes them homologous? What are sister chromatids and are they each considered to be a separate chromosome? Does decondensation of single-chromatid chromosomes, or their replication after M-phase, alter the ploidy of a cell? How does the total number of chromosomes, and the ploidy, in a cell change as it undergoes mitosis (or meiosis)? What is a kinetochore, where on a chromosome does it occur, and what role does it play?

How does mitosis differ from cytokinesis? What structures are often used in a eukaryotic cell to achieve cytokinesis, and are these in any way similar to the structures used during segregation? What is the result if mitosis occurs without cytokinesis right after it? Must the nuclear envelope always break down during mitosis?

What occurs during binary fission in bacteria? For each event you identify ask yourself which phase of mitosis or M-phase of the eukaryotic cycle is most similar to it.

For review see self-quiz questions #3, 7, 9 and 10 at the end of chapter 12.

BIO 108   2010
Day 1, Lecture 3, Title: Mendelian Genetics and Meiosis.
Text Readings: Campbell et al. (2008), chap. 13, and pgs. 262-271.

Objectives:

Know the stages of meiosis. You should be able to work though the stages in figure 13.8 and for a given cell at any given stage be able to determine: The ploidy of the cell at that stage. Whether homologous chromosomes are present or not. Consider what sister chromatids are, how they are made, and what they would become when separated. Does the total number of chromosomes and/or DNA molecules change during meiosis? Be able to relate the appropriate stages of meiosis to the Mendelian principles of segregation and independent assortment. At each stage where they occur be able to describe what is being segregated, and between what things there is independent assortment. How are mitosis and meiosis similar? How do they differ? Crossing over is so much more likely during meiosis that it can be assumed to happen every time, what consequences does this have for events that occur during the second division of meiosis?

Be able to describe the Mendelian model of genetics. What were the new concepts that Mendel devised through his studies? Be able to describe the importance of each of his findings to our understanding of genetics. What were some of the ways that Mendel improved his breeding experiments relative to those done by others before him?

Be able to use the Punnett square to model a cross. Be able to identify the possible gametes, and if given a genotype be able to draw a possible arrangement of the alleles involved on chromosomes as they might exist at a give stage of meiosis.

Given a cross be able to calculate the probability of obtaining a given genotype amongst the offspring. Review the uses of the rules of multiplication and of addition in working problems in probabilities.

[Make sure you note the errors in figure 13.9 of the text. Also note that chapter 14 does a very poor job in describing the principles of segregation and of independent assortment. What it gives concerning segregation is often only true for extremely limited conditions, and in too many cases is inaccurate. See the handout on these principles. What I give you there is more consistent with the way cell biologists view these principles, but be aware that you may encounter views like what the text presents when dealing with geneticists.]

For review see self-quiz questions #5, 6, 8 and 9 of chapter 13. (In my view choices (a), (b), (c) and (e) are all valid answers for question #3, do you see why? Do you see how this relates to the principle of segregation?)

Also for review, see self-quiz quesitons #1-4, 9 and 10 of chapter 14.

3

BIO 108   2010
Day 2, Lecture 4, Title: Post-Mendelian Genetics.
Text Readings: Campbell et al. (2008), pgs. 271-281.

<u>Objectives:</u>

Mendel did not explain everything about genetics. Know the conditions that must be met for Mendelian genetics to apply so that you can describe where Mendel's model of genetics ends and where the model has been expanded beyond his to explain additional observations.

Are all phenotypic traits under strict genetic control? What is an example of an environmental influence on phenotype?

Be able to work with pedigree data to determine if a trait is dominant or recessive and the probable genotypes of ancestors.

How do the following differ: Strict dominant/recessive, incomplete dominance, and codominance? Be able to describe the genetic basis and recognize examples of: Multiple alleles, pleiotropy, epistasis, and polygenic traits. Do these always result in phenotypic ratios different from what would be expected from the Mendelian model? What might be happening at the molecular level that could account for each of these situations? Which of these did Mendel describe and which are post-Mendelian?

What is cytosolic inheritance, in what way does it differ from Mendelian genetics, and in what way is it consistent with Mendelian genetics?

For review see self-quiz questions #5-8, and 11-19 on pgs. 284-285 of your text. (See pg. 283 for some suggestions on how to approach genetic problems, and remember the answers to all these questions are given in the back of the text.)

BIO 108   2010
Day 2, Lecture 5, Title: Chromosome Structure.
Text Readings: Campbell et al. (2008), chapter 15.

Objectives:

What is the chromosomal theory and upon what evidence is it based? Be able to describe sex linkage. Given pedigree data or the results of a cross be able to determine if a trait is likely to be sex linked or not.

Be able to compare the phenotype ratios for the next generation based on the following cross according to the linked versus unlinked genetic model. (Assume each gene codes for a distinct phenotype each in a strict dominant/recessive pattern of its alleles.) Draw how the chromosomes would appear at metaphase I of meiosis for the linked versus the unlinked model.

Cross with genes Unlinked: BbAa X bbaa
Cross with genes Linked: BA/ba X ba/ba

If two genes exist in the same chromosome but are separated by 75 map units will these two genes appear to be genetically linked or not? Will they assort independently during meiosis or not? What is the difference between genetic linkage and physical linkage? Understand the definition of the map unit and how it is not an actual distance. Be able to calculate a map unit from a set of data, or be able to predict what would result in a cross if given a map unit between two genes. Be able to use map units to arrange genes in their proper order on a chromosome.

Why does the percentage of meioses with crossover events in them differ from the map unit? If you are given the percentage of crossover events can you calculate the expected number of recombinant products of meiosis? Be able to describe a crossover event in terms of which chromatids are actually being altered and which are not altered, and relate this to the production of recombinant or non-recombinant offspring.

Be able to describe several ways that the structure of a chromosome can be altered.

Describe X chromosomal inactivation, non-disjunction, and genomic imprinting: Be able to give examples of how each could alter the phenotype of an organism. Consider whether these are consistent with the model of genetics as proposed by Mendel.

For review see self-quiz questions #1-6, and 9-14 of chapter 15.

BIO 108   2010
Day 2, Lecture 6, Title: Fungal Diversity.
Text Readings: Campbell et al. (2008), chapter 31.

Objectives:

Be able to describe the critical differences between the fungal groups Basidiomycota, Zygomycota, and Ascomycota. How are their life cycles all similar? If given a specific example be able to identify common features of that life cycle, including where there are the shifts in ploidy, shared with other fungi. Be able to describe how the structures in which they each create spores differ between fungal groups. How are the life cycles of these fungi similar to that of other eukaryotes, and how do they differ from that of animals?

What are the distinctive features of the Kingdom Fungi that separates it from other eukaryotic kingdoms? What are three different ways in which fungi might acquire food?

To a geneticist what are the advantages of: Examining a trait of a haploid stage of the life cycle compared to working with a diploid stage? The retention of the products of meiosis in an ordered arrangement versus having these products be released? The ability to grow single-celled eukaryotic organisms and make artificial chromosomes that function within them, compared to working with multicellular species for which no artificial chromosomes are readily available?

For review see self-quiz questions #1, 4-6 of this chapter.

BIO 108   2010
Day 3, Lecture 7, Title: DNA as Genetic Material.
Text Readings: Campbell et al. (2008), pgs. 86-89, 305-310.

Objectives:

Be able to describe the studies done by 1) Griffith, 2) Avery and MacLeod and McCarty, 3) Hershey and Chase, 4) Franklin and Wilkins and Watson and Crick, and 5) Chargaff. What evidence did each study provide in support of DNA as a molecule able to carry genetic information? Be able to describe the structure of DNA. Is DNA the sole known carrier of genetic material? What is one example where RNA carries information?

What were some of the major reservations about DNA as genetic material? What alternative was proposed to DNA? Based just on what Griffith found from his transformation experiment describe how it would be possible for the transforming factor to have been DNA or RNA or protein.

What made ^{32}P and ^{35}S good isotopic markers for use by Hershey and Chase? What types of organic molecules does each tend to mark? Are these elements only found in those classes of molecules? What would have been the problem if ^{14}C or ^{15}N had been used as isotopic markers?

What is X ray crystallography, what must one have to do it properly, and what information can be obtained using it?

For review, see self-quiz questions #1 and 5 at the end of chapter 16.

BIO 108   2010
Day 3, Lecture 8, Title: DNA Replication.
Text Readings: Campbell et al. (2008), pgs. 311-319.

<u>Objectives:</u>

Be able to describe the Meselson-Stahl experiment on the pattern of DNA replication. What were they able to show, and how did the evidence they obtained enable them to disprove some of the alternate models of replication?

You should be able to describe the process of DNA replication. All of the items shown in figures 16.13-16.17, and listed in table 16.1, of your text are fair game. Be sure you know the function(s) of each item given there, and the order in which they are used during this process.

What are origins of replication? Does DNA polymerase or an RNA polymerase act earlier at this site? Why should a eukaryotic chromosome have many origins of replication? Be able to contrast replication on leading strands with that done on lagging strands.

At what point in the cell cycle does most DNA replication occur? In a eukaryotic cell which DNA is replicated at variable times and which is replicated mainly at one time in the cell cycle? How is the fact that the DNA was replicated evident in the chromosome structure observed during cell division?

What are telomeres? What role do they play and what problem do they prevent? Why would you expect to find no telomeres in the chromosomes of bacteria or of mitochondria? What enzyme is involved in the production of telomeres, and what would happen to a cell if the expression of the gene for this enzyme was turned off? Do all human cells express this gene all the time?

For review see self-quiz questions #2, 3, 4, and 6 of chapter 16.

BIO 108   2010
Day 3, Lecture 9, Title: Transcription and RNA Processing.
Text Readings: Campbell et al. (2008), pgs. 320-322, 325-336, 347, 356-358, 362-366.

Objectives:

How did Beadle and Tatum use mutants to study the biochemical pathway for arginine in *Neurospora crassa*? In what way could they determine the relative order of each mutation in the pathway? What conclusion did they reach, and in what ways it has been modified in the light of what we know today?

What is the central dogma? Based on what we covered for DNA replication in the previous lecture, what is a modification that should be made to the central dogma?

Be able to describe the process of transcription. What does it make, and what does it need in order to occur? What is the sequence of events that occur during transcription? (Items shown in figures 17.7 and 17.8 should be considered here.)

What are the three major types of RNAs made in cells? What are two minor types of RNAs? Be able to describe the functions of these different types of RNA.

In eukaryotic cells processing of the RNA is essential. What are ways in which RNA is often altered? What are the differences between a primary transcript and a mature messenger RNA? Be able to identify and describe the function(s) of the regions of mature mRNA shown in fig. 17.9. What possible role(s) might RNA processing serve? What are snRNPs and where would you expect to find them in a eukaryotic cell, and what functions do they carry out?

What is RNA interference, what enzymes are often involved, and how does this process differ from RNA processing? Be able to describe several other levels of control of gene expression that are typically seen in eukaryotes.

For review, see self-quiz questions #1 and 4 of chapter 17, and self-quiz question #8 of chapter 18.

BIO 108   2010
Day 4, Lecture 10, Title: Translation.
Text Readings: Campbell et al. (2008), pgs. 328-331, 337-344, 346-348.

Objectives:

Assuming that the genetic code will be available to you, be able to take a sequence of DNA or RNA and determine what sequence of amino acids they should code for in a protein. Do all genes code for proteins? Other than proteins, what are some other gene products and what are some of the functions these other gene products carry out?

Be able to describe the process of translation. What items are needed and what happens during each stage (i.e. initiation, elongation, termination)? What molecule actually catalyzes the formation of peptide bonds? (Items in figures 17.17, 17.18, 17.19 are good to go over here.)

What are the important structural features of tRNA? What role does tRNA play in translation? With what other molecules must it interact covalently and non-covalently? How are tRNAs activated for use? What are the important structural features of mRNA that are essential for it to carry out its functions? And what functions do rRNAs carry out? Do only tRNAs interact with codons? What are release factors?

Proteins can be created in the cytosol, in some organelles, or a protein may be a membrane protein, or targeted for secretion, or to be moved into other cellular compartments. How can this protein sorting be achieved? What is an SRP and what is it made up of? What role does it play in translation? Does the translation of all proteins involve an SRP? If you have the DNA sequence of a part of a gene that is complementary to the coding region of mRNA what would you need to know if you wish to determine if it will lead to the formation of a membrane protein versus a cytosolic protein?

For review, see self-quiz questions #2, 3, 5, 7 and 8 of chapter 17.

BIO 108   2010
Day 4, Lecture 11, Title: Control of Gene Expression I.
Text Readings: Campbell et al. (2008), pgs. 346-347, 351-358, 362-366.

Objectives:

Be able to describe the options shown in fig. 18.6 for control of phenotypic expression in eukaryotes. Be able to describe how chromosome condensation, RNAi, riboswitches, and the ubiquitin systems fit into the stages shown in this figure.

What options of control of expression differ in bacteria versus in eukaryotes? What role does supercoiling play in the control of bacterial gene expression? What is the benefit to a bacterium in having different levels of control of expression? What would be a disadvantage of having all genes expressed all the time and equally? Be able to describe reasons and mechanisms for controlling and altering rates of gene expression.

Be able to describe the operon, its component parts, and the consequences of having several genes' expression controlled by a single promoter. Consider advantages and disadvantages of organizing genes into operons. What are the names of the various types of proteins that can interact with DNA near or in the promoter of an operon that influence the initiation of transcription? Be sure you understand how the role of a repressor protein, and expression of its gene, relates to the operon on which it acts.

The examples of the *trp* and *lac* operons are worth understanding. How does an inducible operon differ from a repressible operon, while at the same time both are examples of negative control? What is the difference between negative and positive control? Can you describe positive control of the *lac* operon? How does CAP influence the ability of RNA polymerase to initiate transcription? What are the essential preconditions for positive control of the *lac* operon to occur?

For review see self-quiz questions #1, 3, 8 and 9 of chapter 18.

BIO 108   2010
Day 4, Lecture 12, Title: Control of Gene Expression II.
Text Readings: Campbell et al. (2008), pgs. 300-301, 320-323, 356-366, 432-442, 939-940.

Objectives:

Be able to describe several ways that control of gene expression in prokaryotes often differs from its control in eukaryotes.

How does the structure of a chromosome influence gene expression? What is an advantage of being able to highly condense eukaryotic DNA? What is the role of histones? Be able to describe epigenetic changes that influence gene expression. Would DNA tend to be more methylated in euchromatin or in heterochromatin?

Be able to describe the role of transcription factors in bacteria versus in eukaryotic gene expression. What must transcription factors be able to do to function normally? How is a eukaryotic gene and its transcriptional control similar and how is it different from a bacterial operon and how its transcription is controlled?

How do distal control elements influence gene expression in eukaryotes? What are the roles of activators and enhancers? Be able to describe in a general way how a protein can interact with DNA in order to alter gene expression.

What are mechanisms that might result in the amplification of the gene copy number in prokaryotes and in eukaryotes? What is an advantage of such a process, and what is a cost? How might gene families be produced?

Exon deletion in the maturation of immunoglobin genes is a good example of genetic deletion as a form of control of gene expression. Why can not gene deletion be a more common form of control? What would be the consequence for offspring if it were more common? Are these changes in the immunoglobin genes inherited or not?

For review, see self-quiz questions #2 and 4 of chapter 18, and self-quiz questions #4 and 5 of chapter 21.

BIO 108   2010
Day 5, Lecture 13, Title: Viruses.
Text Readings: Campbell et al. (2008), chapter 19, and fig. 6.2.

Objectives:

Relative to the size of a bacterial cell, like *E. coli,* or to a typical human skin cell, how large are viruses? How do viruses compare in size to mitochondria, or to membrane-spanning proteins?

Viruses come in various forms. Be able to describe some of the structural and compositional options often seen in different types of viruses. How does an RNA virus differ from a DNA virus? How do these differ from a retrovirus? What are bacteriophages? Some viruses carry unique enzymes with them or have genes to code for them. What are some of these enzymes and what function does each of these enzymes carry out?

What are the differences between the lytic and lysogenic viral cycles? What are prophages, and what role do they play in which of these cycles? What enzyme activities would be needed for a lysogenic virus to integrate its DNA into the host cell's DNA?

Viruses that infect human cells have to deal with our immune system. What are some ways in which viruses can get around the defenses of their host? What defenses do bacterial cells have against viruses that attack them?

For review, see self-quiz questions #1-4 and 7-8 of chapter 19.

BIO 108   2010
Day 5, Lecture 14, Title: Prokaryotic Diversity.
Text Readings: Campbell et al. (2008), pgs 551-553 and Chapter 27.

<u>Objectives:</u>

What are some distinguishing features between the Archaea and the Bacteria and the Eukarya?

As the dominant life forms on the planet it is worth knowing the range of ecological distribution of prokarotes, the types of metabolic roles they play, and their general life strategies.

Be able to describe the structures found in bacteria, and the roles they play, such as: The nature of their cell walls, and capsules. Be able to describe the differences between gram positive and gram negative bacteria, the structural features of their chromosomes, the shapes bacterial cells often show, the function of bacterial spores and of their flagella and pili. How is the bacterial flagellum different from the eukaryotic flagellum? How can bacteria protect themselves from viruses?

Table 27.2 presents the stereotypical view of bacteria as lacking multicellularity or any sort of cellular specialization, always having circular chromosomes, lacking internal organelles, etc... You should know this stereotype, but also be able to describe exceptions to some of the features given in this table.

What are plasmids and what benefits do bacteria get from being able to form plasmids? What must a plasmid be able to do if it is going to be useful? The process of conjugation is one means that bacteria have which differs greatly from how eukaryotes carry out genetic recombination. Be able to describe this process. How can plasmids be involved in this process? Consider how the use of plasmids and conjugation can promote cellular specialization and make individuals more adaptive for conditions in which they live. Be able to describe the process of transduction and how it can alter the genetic combination in a bacterial cell. How are aspects of transduction and conjugation similar to the crossing over seen during meiosis?

For review, see self-quiz questions #1, 2, and 4 of chapter 27.

BIO 108   2010
Day 6, Lecture 15 , Title: Recombinant DNA I.
Text Readings: Campbell et al. (2008), chapter 20.

Objectives:

The techniques of Southern Blotting, electrophoresis, DNA sequencing, and the use of restriction enzymes are all essential for the finding a candidate gene from isolated DNA. Be able to describe the use of these techniques and what is needed to carry each out. What is the function of the deoxy-DNA triphosphates in the Sanger method?

What must be known to make a good probe for DNA hybridization? If you have knowledge of the amino acid sequence of a protein how could you propose a possible probe for its gene? What state must the DNA be in for it to interact with a good probe, and how do you get it into that state? Which techniques make use of some aspect of DNA hybridization?

What are some different forms of gel electrophoresis used to study DNA, and what is an advantage of each type?

Restriction enzymes are used by bacteria for what purpose? When we use these enzymes for our purposes what do they give us the ability to do? What is recombinant DNA?

Be able to describe the process of PCR and know what is needed to carry it out. What must be known before this process can be used? Be able to relate this technique to other ways we will cover in later lectures for the production of copies of a desired section of DNA.

For review, see self-quiz questions #1, 4, and 7 of chapter 20.

BIO 108   2010
Day 7, Lecture 16, Title: Fertilization.
Text Readings: Campbell et al. (2008), pgs. 206-207, 806-807, 812-815, 997-1003, 1021-1025, and figures 46.12 and 46.15.

Objectives:

What role do nurse cells play in the growth of the animal primary oocyte before fertilization? What is the state of the genetic material in the primary oocyte during this time? What effect does this have on gene expression, and how does this relate to the need for nurse cells? What are polar bodies? How many are made, and what state are the chromosomes in in each as it is formed?

Know the structures found in sperm and secondary oocyte cells that are critical to fertilization (Figures 46.12, 47.3, and 47.5 may help here).

What must happen to an animal sperm cell before it can fuse with a secondary oocyte? What roles do capacitation and the acrosomal reaction play in preparing a sperm cell for plasmogamy?

Fertilization can be divided into the substages of plasmogamy and karyogamy. Be able to describe these substages in the process of animal fertilization.

How does the secondary oocyte select sperm of its own species? How do the slow and fast blocks to polyspermy differ and how does each work? What is the role of the *zona pellucida*? Be able to describe the steps in the acrosomal and cortical reactions. How are the processes of the acrosomal and cortical reactions similar to what occurs in a presynaptic neuron? Other than its genetic material, what do many sperm of many species also contribute to the resulting zygote?

Consider the benefits and costs of the use of fertilization in a species' life cycle: Under what conditions might asexual reproduction be favored? Be able to discuss the use of cell signaling systems by animals, and compare this to systems used in fungi and plants that attempt to undergo mating.

For review, see self-quiz question #8 of chapter 46, and #1 of chapter 47.

BIO 108   2010
Day 7, Lecture 17, Title: Angiosperm Life Cycle.
Text Readings: Campbell et al. (2008), pgs. 252-253, 600-606, 618-621, 625-634, 801-811.

Objectives:

Compare and contrast the life cycle of animals with that of flowering plants. Be able to indicate the ploidy and names of each stage in the life cycles, and to identify the sexual reproductive structures of flowering plants. Be sure that you can find and understand the roles of each of the following in the angiosperm life cycle: sporophytes, gametophytes, gametes, spores, sporangia, zygote, embryo. Be able to contrast homosporous and heterosporous plant life cycles.

Unlike animals where meiosis and fertilization are closely associated in the life cycle, plants separate these processes of sexual reproduction to different stages of the life cycle. Be sure you understand how both meiosis and fertilization are sexual and reproductive acts in the plant life cycle. In flowering plants what structures engage in the meiotic part of sexual reproduction, and what structures are involved in the fertilization part of sexual reproduction? Describe the process of double fertilization, including the roles played by the synergid and central cell. Understand the origin and function of the endosperm in the developing seed.

Be able to describe the parts of a perfectly complete flower and their major functions, versus that of an imperfectly incomplete flower.

Be able to describe how the angiosperm male gametophyte is formed, what cells it contains when mature, and the function(s) associated with it in the life of flowering plant species.

Correctly place and identify angiosperm ovule structures and functions it carries out at various stages of its development from it role as a sporangium to its being a seed.

Compare and contrast the functions of the ovarian wall when it first surrounds immature ovules, versus later when it surrounds mature seeds, in terms of what the ovarian wall might have to be doing at each of these different points of the life cycle.

Be able to describe various solutions that plants have evolved to address the problems of inbreeding, achieving pollination, fertilization, and dispersal of their species.

Figures 30.10, 38.3, 38.5 and 38.7 cover most of the structures and processes described in this lecture and may be good ones to review.

For review, see self quiz questions #1-3 of chapter 30, and questions #2, 3, and 4 of chapter 38.

BIO 108   2010
Day 7, Lecture 18, Title: Plant Kingdom and Evolution.
Text Readings: Campbell et al. (2008), chapter 29, and pgs. 618-625.

Objectives:

Have an appreciation of the time frame of events in plant evolution. When did plants appear, and if there were no plants on the land earlier in the history of life on earth who did the photosynthesis? What were the major photosynthetic groups on earth before plants evolved? What are some major non-plant groups that do photosynthesis today?

You should know the fundamental characteristics that now are used to define the plant kingdom. (Comparing plants to an out group, such as some members of the green algae, might be helpful.)

The life cycle of plants should be clear to you, and given a representation of stages of the life cycles of mosses, ferns or pines you should be able to identify the stages in such a figure. Be sure that you could pick out in such a figure, and understand the roles of: sporophyte, gametophyte, gametes, zygote, spores, sporangia, archegonia and antheridia.

Be able to describe the influence of selection for dispersal and desiccation tolerance on the evolution of plants. What features of mosses, ferns, and pines promote their dispersal, or promote their avoidance of desiccation? Be able to identify the stages that are most sensitive to desiccation, and describe how each plant group copes with this problem. Be able to relate the ability of each of these plant groups to tolerate or avoid desiccation to the type of habitat where each is found. How do these plants differ in how they achieve dispersal?

Compare and contrast a homosporous versus a heterosporous life cycle in terms of the costs and benefits of each to a plant that has either trait. What is pollination, and how does it differ from dispersion and from fertilization?

The ovule, and how it develops into a seed, is a process you should be able to describe.

For review, see self-quiz questions #2, 3, 4, 6 and 8 of chapter 29, and questions #4-6 of chapter 30.

BIO 108   2010
Day 8, Lecture 19, Title: The Immune System.
Text Readings: Campbell et al. (2008), chapter 43.

<u>Objectives:</u>

Describe several mammalian non-specific defenses against infection. Which mechanisms depend on the direct action of cells and which are more indirect? What is involved in the inflammatory response? Describe the types of cells involved, the signals sent, and the responses made as a result of infection. What role do TLRs play? What is the complement system and what effect might it have on an infecting bacterium versus on an infecting virus?

What are the types of cells found in the B-cell system and what types are found in the T-cell system? Be able to describe each cell's function(s). How do these systems detect foreign items in the body, and where do they look? Be able to describe receptors used by each system, and their responses once activated. Describe how the immune system is kept from creating antibodies and T-cells that bind to self-antigens, or from destroying healthy cells. Be able to describe the roles of the lymphatic system, lymph nodes, and thymus in the immune system functions.

What is the structure of a typical antibody (IgG)? How is the type of antibody to be made determined and how is the DNA altered during this process? What is clonal selection, and what types of immune cells are involved in it or made by it? What effects can antibody binding have on a pathogen or a toxin? Be able to describe and contrast important features of the primary and secondary immune responses.

The interactions of MHCs and CD surface proteins and TCRs are important to know about so that the interactions of the T cell system can be understood. Which cell types have MHCI, and which MHCII? What cell types have CD4, and which have CD8? What is the purpose of having two parallel sets of these proteins? What do different types of T cells do as their major functions?

What is an antigen presenter cell and what role does it play in the activation of the immune system? What are cytokines and what role do they serve? Be sure you understand how the B-cell system and innate immune cells can interact with the T-cell system.

For review, see self-quiz questions #2, 3, 5 and 6 of chapter 43.

BIO 108   2010
Day 8, Lecture 20, Title: Animal Development I.
Text Readings: Campbell et al. (2008), pgs. 1025-1044.

Objectives:

We have three model vertebrates to consider: frogs, chickens, and humans. Be able to compare them to each other at the early stages of embryogenesis and contrast the patterns they show as they carry out gastrulation and neurulation.

Given a frog embryo be able to identify the early anterior/posterior axis and the dorsal/ventral axis of the embryo that will grow from it.

Describe how experiments that divide up a frog zygote, and experiments that track the developmental fate of early cells, are consistent with the presence of cytosolic factors. Be able to describe the use of transplantation experiments to study embryonic organizing centers. What are examples of organizing centers and how is it proposed that they operate: What molecular systems are necessary?

At what point in animal development are there distinct tissues present, and what are their names? What is the difference between determination of cell fate and differentiation of cell type? At what point do the first organs begin to form?

The frog embryo has lots of yolk. Where does the yolk end up in the gastrula and neurula stages of a frog? Where does the yolk end up in similar stages of the chicken embryo? How does the amount of yolk influence early cleavage and the shape of the blastula? How do human embryos obtain nutrients for growth? In each of these three vertebrates how does the embryo manage to gain access to food whether in the yolk or not?

Know the structures in the amniotic egg, and be able to compare the function and role of these membranes in chickens versus humans (figs. 47.13, 47.15, and 47.16 may be helpful here).

For review, see self-quiz questions #3-8 of chapter 47.

BIO 108   2010
Day 8, Lecture 21, Title: Animal Development II.
Text Readings: Campbell et al. (2008), pgs. 223-225, 366-373, 412-416, 442-447, 757-761.

Objectives:

Is all development influenced by the products of the genes within a single cell, or do gene products from some cells ever influence the determination and differentiation of other cells? What are examples of intracellular factors and extracellular factors that influence cell fate? Was it intracellular or extracellular factors that made cloning of animals so difficult to achieve? You should be able to describe examples of the role of such factors in differentiation, such as the role of bicoid in fruit flies, and how vulva induction is achieved in nematodes. Consider what role apoptosis can play during the development of an organism.

What are maternal effect genes, in which generation do they have effects, and whose genotype is having the influence on development in this case? Be able to describe the bicoid system as an example of a maternal effect system.

Be able to describe generally what happens to alter gene expression during cellular determination and differentiation. What is totipotency, and what would a cell that has this trait be able to do? Describe examples of experimental evidence of totipotency in adult animal and plant cells.

Controlling patterns of gene expression can involve information transduction systems. Can you give examples of several developmental situations in which there are signals passed between cells, suggest some hypothetical transduction systems that might be involved, and describe the likely results they would have?

Homeotic genes are found in animals, plants, and other eukaryotes. What are homeotic genes, and what might they control? Across animal species are the homeotic genes similar or different, and how would you account for this? What are homeoboxes and homeodomains, and in what types of molecules are they found?

Describe what changes you would look for, and in what types of molecules you would look, if you wished to study first the determination and then the differentiation of an adult cell type from an embryonic stem cell.

For review, see self-quiz questions #5, 6, and 7 of chapter 18. Self-quiz questions #6 and 8 of chapter 21. And self-quiz question #10 of chapter 35.

BIO 108   2010
Day 9, Lecture 22, Title: Phylogeny and Systematics.
Text Readings: Campbell et al. (2008), chapter 26.

Objectives:

If given two simple phylogenetic trees you should be able to evaluate which is most parsimonious. How is parsimony used to choose the "best" phylogenetic tree? Be able to use a phylogenetic tree to evaluate the relatedness of the taxa shown in it.

There are some terms worth knowing: In group, out group, analogous, homologous, shared ancestral characters, shared derived characters, monophyletic, polyphyletic, paraphyletic, clades and cladograms. Be able to use each of these terms.

What must be known in order to carry out a cladistical analysis? What assumptions are made in the assembly of a cladogram? Given an appropriate data set, be able to construct and use a cladogram.

What are some benefits and pitfalls of using molecular data in classification? What does information from either molecular data or fossils sometimes allow to be added to a hypothetical phylogeny? What assumptions are made in the use of "molecular clocks," and what information can be obtained with their use? What makes a set of traits homologous versus analogous? Be able to identify a set of analogous traits produced by convergence, and a set of homologous traits produced by divergence.

What is the difference between vertical gene flow and horizontal gene flow? What is a process or event that is an example of each of these? What is the consequence of each of these on attempts to reconstruct evolutionary relationships?

For review, see self-quiz questions #1, 3-9 of chapter 26.

BIO 108   2010
Day 9, Lecture 23, Title: Mutations.
Text Readings: Campbell et al. (2008), pgs. 297-302, 316-318, 344-346, 356-363, 373-377,
      432-442, 495-496, and 576-577.

Objectives:

Be able to describe the differences between such point mutations as: missense, nonsense, silent, and frameshift mutations. How important might each be in terms of effects on the protein that will be made? Are these mutations in genes or non-genetic regions of the DNA? Are they in introns or in exons?

What is the typical rate of mutational changes seen in eukaryotes? What consequence does this have for the number of cell divisions that can be done in a multicellular organism?

What are transposons and of what parts are they composed? What is the difference between a simple and a composite transposon? What enzymatic activities are needed for transposition to take place? What could be some possible consequences of transposable elements on the control of gene expression?

What are changes that can happen in a chromosome, other than in the expressed genes themselves, that could alter gene expression? What are ways in which the structure of a chromosome can be altered?

Mutations can lead to cancer. Based on what is covered in the text (see pgs. 373-377) be able to describe changes in the controls of several types of genes that typically lead to cancer.

How do the processes of endosymbiosis and polyploidy promote the production of new genetic combinations? Do these processes occur within an individual, within a species, or can they occur between individuals of different species? Some describe organisms like ourselves as chimera (a mixture of several different species). What arguments support this view?

Be able to argue for how mutations are on one hand costly to an individual, but they are also often ultimately beneficial to a species, and helpful in promoting the process of speciation.

For review, see self-quiz questions #3, 4, and 5 of chapter 21.

BIO 108   2010
Day 9, Lecture 24, Title: Recombinant DNA II.
Text Readings: Campbell et al. (2008), pgs. 561-564, and chapter 20.

Objectives:

Be able to describe several options for getting foreign DNA into a host cell. What types of vectors are available that can carry the DNA into the cell? What various methods can be used to get DNA into a cell? Be able to describe some advantages and disadvantages of each method and vector type. How is the uptake of the DNA detected? Be able to describe the use of reporter genes (see fig. 20.4), how each alters the phenotype of the cell that expresses them, and what sort of information each type of reporter gene provides us.

Getting the DNA into the cell is not enough, what else must happen? What could go wrong if you attempt to express a eukaryotic gene in a bacterial cell? What is needed to carry out Northern and Western blotting techniques, and what does each of these types of blotting tell you compared to what Southern blotting could tell you?

What are YACs and BACs and what do they allow molecular biologists to do that simple transformation or plasmid-based systems can not?

What is a DNA library, and for what it is used? How would it be prepared? What can a DNA microarray allow you to do, and how does it detect DNA? What are some advantages and disadvantages of working with DNA versus mRNA as the starting material? What is cDNA, and what would it tell you that you could not easily determine if you worked directly with DNA?

What is gene "therapy" and what is it attempting to do, and what are some of techniques upon which it depends?

For review, see self-quiz questions #2, 8, 9 and 12 of chapter 20.

BIO 108   2010
Day 10, Lecture 25, Title: Darwinian Evolution.
Text Readings: Campbell et al. (2008), chapter 22.

Objectives:

Be able to describe the Darwinian view of evolutionary theory. What conditions must be met, and what are some assumptions made, by Darwin's model of speciation? What did Darwin view as the major mechanism leading to speciation? What predictions follow from Darwinian evolution that would not have been expected otherwise?

What were some of the biological and geological views that preceeded Darwin's model and how did they influence his model? What ideas from geology and economics did Darwin incorporate into his model? What were some of the scientific views before Darwin's time that limited thinking about the formation of new biological species? What were some concepts that became evident once Darwin's model was produced that would not have been much considered before?

Understand the argument that while species have and continue to evolve our biological models of that evolution are incomplete. What were some major aspects of Darwinian evolutionary theory that can be argued to have been incomplete or strongly in doubt at the time he proposed it?

For review, see self-quiz questions #1, 4, 5, 6 of chapter 22.

BIO 108   2010
Day 10, Lecture 26, Title: Invertebrates.
Text Readings: Campbell et al. (2008), chapters 32 and 33.

Objectives:

What characteristics are typical of all members of the animal kingdom? Know the characteristics of the following groups: "Porifera", Eumetazoa, Bilateria, Lophotrochozoa, Ecdysozoa.

In terms of the traits that are shared, be able to describe the reasoning for assembling phyla into the Lophophozoa and Ecdysozoa clades. What is ecdysis?

Be able to describe several functional issues that all invertebrates must address in order to be successful. What issues must terrestrial invertebrates deal with that are not major problems for marine invertebrates? What are several advantages of being small in size? What are several advantages of being larger in size? What functional issues does each option help address?

Be able to contrast traits typical of protostomes and deuterostomes, and identify examples of animals that belong to each of these groups, and animals that belong to neither of these groups. What characteristics arose in animals long before these groups, and are these groups monophyletic or not? (Looking at figure 32.11 may be helpful here.)

Contrast the characteristics found in the following groups of animals and how they meet the needs of circulation, waste removal, gas exchange, movement and mechanical support: sponges ("Porifera"), hydra (Cnidaria), planaria (Platyhelminthes), earthworms (Annelida), clams and squid (Mollusca), and arthropods (Arthropoda). For these animals know the distinctive traits found in each.

Insects are arthropods that are very successful terrestrial invertebrates. How do insects manage to be such active organisms and yet have an open circulatory system? Being so small, how do they manage to avoid drying out?

What is a coelom, what are examples of it, and what advantage does it give to an organism? What functions can a coelom help to address?

For review, see self-quiz questions #1 and 2 of chapter 32, and questions #1, 2, 3, and you might try doing #7 just for the phyla we have covered, of chapter 33.

BIO 108   2010
Day 11, Lecture 27, Title: Bioassays.
Text Readings: Campbell et al. (2008), pgs. 583-584 (malaria), 798 (dodder), 821-841.
Article Readings: (PDF versions posted on the course web site.)
  Runyon J.B., M.C. Mescher, C.M. DeMoraes- 2006-Volatile chemical cues guide host
    location and host selection by parasite plant- Science 313: (9/29) 1964-1967.
  Lacroix R., W.R. Mukabana, L.C. Gouagna, J.C. Koella- 2005-Malaria infection
    increases attractiveness of humans to mosquitoes- Public Library of Science
    Biology 3: (#9) e298, 4 pages.

Objectives:

Growth assays and the Ames test are examples of bioassays. How specific is each of these bioassays? Is specificity always desired? Can you think of examples of when it might be an advantage to have a broader test? Be able to describe how the Ames test operates, and what it is designed to detect. From your reading, be able to identify several plant responses that have been studied by the use of bioassays. Be able to describe how a growth response to a hormone is different from a growth response due to the supply of more nutrients.

The evaluation of threshold, linear response, and saturation levels of response are critical aspects of quantitative bioassay systems. What can cause a treatment to result in a response that is below threshold, or to its being above the saturation level? Be able to describe the state of the receptor when at any of these three levels of response.

Be able to describe the studies done by Lacroix et al. (2005) and Runyon et al. (2006) in terms of the experimental questions being addressed, the methods used, and the important results and conclusions reached. In each case what aspects of the experimental design helped to reduce the effects of extraneous variation on the results? In each of these studies what would be a next logical step to take?

For review, see self-quiz question #1 of chapter 39.

BIO 108   2010
Day 12, Lecture 28, Title: Post-Darwinian Evolution.
Text Readings: Campbell et al. (2008), chapter 24 and pgs. 483-484, 525-531.

Objectives:

The term "species" is a concept that has some flexibility. How does the definition of this term depend on the circumstances, and what are the reasons for these variations? Be able to describe situations in which it would be appropriate to use different species concepts.

Darwinian evolution is a model which has been modified. What are some of the modifications that the current biological evolutionary model incorporates that Darwin's model of speciation did not take into account? Does the refinement of the model result from doubt about the fact of new species being formed, or does it reflect an acceptance of new observations concerning the mechanism(s) of speciation? Be able to describe some of the important new observations and mechanisms.

What is punctuated equilibrium, and what new claims does it make? How might gene flow occur between species? What barriers must be overcome to result in the formation of a new eukaryotic species? Be able to compare allopatric speciation and sympatric speciation. Which one can be more rapid? Which one requires more geographic space? Are all speciation mechanisms dependent on natural selection and on long periods of time?

For review, see self-quiz questions #1-6 of chapter 24.

BIO 108   2010
Day 12 , Lecture 29, Title: Population Genetics.
Text Readings: Campbell et al. (2008), chapter 23.

Objectives:

Given a set of genetic data of a population you should be able to determine the allele, genotype, or phenotype frequencies, and if given sets of data taken at different times you should be able to determine whether or not the population is in Hardy-Weinberg equilibrium. What are some typical exceptions to the Hardy-Weinberg model, and which of these exceptions are not accounted for by the model of evolution as it was originally proposed by Darwin? Which of these exceptions are often adaptive, and which produce random changes that may or may not be adaptive?

In terms of a population's genetics what must happen in order for that population to evolve? According to the Hardy-Weinberg model what are several ways in which a population can carry out this evolution, which ways are more rapid and which will require the most time, which could make the largest changes in the least amount of time?

For review, see self-quiz questions #1-5 of chapter 23.

BIO 108   2010
Day 12 , Lecture 30, Title: Pollination Ecology.
Text Readings: Campbell et al. (2008), pgs. 630-632, 804-805, 1198-1203.

Objectives:

Describe several strategies that flowering plants use to achieve pollination. What modifications to what parts of the plant are associated with each of these strategies? What costs to the plant does each strategy impose? Be able to describe several of the benefits to those animals that act as pollinators. What do some of these animals gain? Gain an appreciation of examples of plant/animal interactions. Be able to classify pollinator/plant interactions in terms of those given in the table on pg 1219 (in the review section of chapter 54).

Is the pollination of flowering plants fully dependent on animals or is it every achieved by abiotic means? How would the flowers of such plants differ from those that use pollinators? What are several examples of plants that use abiotic pollination?

Be able to describe the differences between pollination and fertilization, and the differences between pollination and dispersal as seen in the flowering plants.

For review, see self-quiz question #1 of chapter 38.

BIO 108   2010
Day 13, Lecture 31, Title: Origin(s) of Life.
Text Readings: Campbell et al. (2008), pgs. 58-59, 507-525.

Objectives:

What energy sources were available to promote the formation of complex organic molecules from inorganic constituents? How might polymerization have been achieved?

Miller and Urey did several experiments using what they estimated to be conditions found on the early earth. What did they do and what did they conclude? What evidence supports their estimates of the early conditions on earth?

What are hydrothermal vents and what advantages do they offer as a site for the early origins of life? Having catalysts is essential, but the catalysts must be capable of coupling reactions.   Why is this so, and what types of reactions should they be able to couple? What are ribozymes? What properties do they have that make them an interesting candidate as an early genetic element?

How easily do vesicles form? What are advantages and disadvantages of having membranes present during the formation of life?

Replication and reproduction are seen in life, and are also seen in some non-living systems. How do these two processes differ? Must replication and reproduction always be tightly coupled, or can they occur separately? What are examples of abiotic systems that show replication, or reproduction, and yet are not alive?

Abiotic chemistry is essential to form many of the molecules needed for the origin of life. Should a biologist argue that these abiotic processes must be accounted for by the biological theory of evolution? How do biologists account for the failure to find life originating spontaneously today? How might this argument also account for the observation of just one clade of life on earth today rather than several distinct clades of life?

BIO 108   2010
Day 13, Lecture 32, Title: Ecology, abiotic factors.
Text Readings: Campbell et al. (2008), chapter 52, and pgs. 1226-1228.

Objectives:

How does the distribution of solar radiation differ across the Earth's surface, what causes this distribution, and what consequence does this have for temperature and regional climate? How does this influence the distribution of rainfall and of the trade winds?

How does the movement of water in the oceans act in a manner analogous to that of the movement of air? How is the flow of the gulf stream powered? What powers the movement of the air to create the trade winds?

How do geographic factors alter rainfall and regional temperatures? Describe the rainshadow effect, and if given a map showing geographic features and prevailing winds be able to identify areas of higher and lower rainfall.

In what way does the fact that the Earth is tilted contribute to the variation in climate? What role does the variation in the local climate play in determining the strategies used by species that attempt to survive in an area?

Describe how minerals can be locked up in a lake. What keeps the nutrient-rich water in the bottom of a lake from mixing well with the surface water? How is this similar to nutrient conditions in some parts of the ocean? How does this compare to how minerals are often locked up in tropical rainforests?

Be able to describe the following biomes, which are presented in figure 52.21, in terms of abiotic factors that have a major influence on each of them: Tropical forest, desert, savanna, coniferous forest, temperate broadleaf forest, and tundra.

For review, see self-quiz questions #2-4, 6, 8-9, and 12 of chapter 52.

BIO 108   2010
Day 13, Lecture 33, Title: Population Ecology.
Text Readings: Campbell et al. (2008), chapter 53.

<u>Objectives:</u>

Contrast R- and K-selected strategies in terms of not just population growth but also the characteristics of a species that uses each strategy. What features of the environments in which these species live may have resulted in the selection for either of these strategies?

Understand the meaning of the terms in the exponential and logistic growth equations. If given values for these terms be able to calculate an estimate of future population change.

What assumptions are made, and what would you wish to know, before attempting to do a mark and recapture study of an animal species? Be able to use sample data to estimate total population size.

How does the age structure of a population relate to the strategy a species adopts? How would the age structure differ between a population that is growing versus one that is shrinking? Does fecundity stay the same with age? What are semelparity and iteroparity, and how would a population's age structure likely differ for each of these cases?

What are some density-dependent limits on population growth? What advantages do individuals of a species gain from living in groups? What advantages do individuals gain from living in a more scattered pattern?

For review, see self-quiz questions #1-5, 7-9, of chapter 53.

BIO 108   2010
Day 14, Lecture 34, Title: Community Ecology.
Text Readings: Campbell et al. (2008), chapter 54.

Objectives:

Be able to define and give examples of various types of interspecific interactions such as commensalism, mutualism, predation, and competition. Be able to describe examples of competitive exclusion and relate this phenomenon to speciation.

Describe the concept of niche, and differentiate between fundamental and realized niche.

How can species interactions help to determine the types of species present in a community? Would the occurance of periodic strong disturbances promote or degrade these interactions? How could such disturbances promote, or inhibit, the presence of a species in an area?

Describe the concept of a keystone species and give an example of it. What is a facilitator species and what does it do?

How do primary and secondary succession differ? Give examples of each.

In what way is the diversity of species in an area dependent on the size of the area and on the amount of disturbance suffered by the area? What other factors might influence the diversity of species found in an area? What uses can be made of the information given in a species/area curve for a community?

Is a biological community a stable entity? What factors promote its stability? What factors make it unstable? What characteristics would you wish to know about if you wished to be able to describe the composition, interactions, and functions of a biological community?

For review, see self-quiz questions #1, 2, 3, 5 and 8 of chapter 54.

BIO 108   2010
Day 14, Lecture 35, Title: Ecosystems.
Text Readings: Campbell et al. (2008), chapter 55.

Objectives:

Describe the flow of energy and matter through an ecosystem in terms of the producers, consumers, and detritivores. How do the patterns of flow of energy and matter differ, and what processes link the trophic levels? What role do the production efficiencies play in determining the number of individuals that can be supported at higher levels on the energy pyramid? What is biomagnification?

What role does the rate of turnover of primary producers have on the rate of response of an ecosystem to disturbances?

What evidence is there that nutrients are often limiting to the productivity of an ecosystem? Be able to describe specific experimental evidence. Even though the open ocean has plenty of water why does it often show a low net primary productivity when compared to a coastal marsh? What is often present in the marsh that is lacking in the mid-ocean areas?

Be able to describe the nitrogen and carbon cycles. What are the processes of nitrogen fixation, ammonification, nitrification, and denitrification? What process does carbon fixation, and what processes release carbon from organic matter? Where do these processes fit into each cycle?

How is the phosphate cycle different from that of carbon or nitrogen? Why does this have less consequence for ocean ecosystems compared to terrestrial ones?

Be able to describe how the rate of flow of energy and various minerals would be expected to differ in an arctic ecosystem from that in the rainforest, or in various aquatic ecosystems. What biotic and/or abiotic factors account for the differences?

For review, see self-quiz questions #1-3, 5-7 of chapter 55.

BIO 108   2010
Day 14, Lecture 36, Title: Conservation Ecology.
Text Readings: Campbell et al. (2008), pgs. 1236-1242, and chapter 56.

Objectives:

Habitat fragmentation causes the loss of habitat and an increase in "edges" or transition regions between habitats. What influence does this have on efforts to conserve species? Be able to relate species/area relationships to attempts at conservation.

What knowledge of the ecological characteristics of a species need to be known in order to preserve it as well as the ecosystem in which it lives? Understand the concepts of MVP and N_e, and be able to relate them to efforts to maintain a small population. Do any species depend upon disturbance in order to survive? Would such disturbance be left to occur by chance, or must humans often supply it? What are keystone species, and what role might they play in conservation efforts?

Be able to describe the greenhouse effect and how it drives global warming. What gases are greenhouse gases? Be able to describe other specific regional/global environmental changes that threaten the survival of either individual species or entire ecosystems.

Gain a sense of the rate of species extinction, and various causes of extinction.

Decide what you feel you should do in the face of such challenges to the quality of human existence.

For review, see self-quiz questions #1, 4, 6, 7 and 8 of chapter 56.

BIO 108   2010
Day 1, Lecture 1, Title: Eukaryotic Life Cycles.
Text Readings: Campbell et al. (2008), pgs. 248-253, 638-640, 812-813, 997-1000, and figures
13.5, 13.6, 28.11, 28.22, 29.5 (Alternation of Generations), 29.13, 31.5, 31.19.

(First course intro, and course business...)

Topics to cover:

      Advantages and Costs of a Sexual Life Cycle
      Common Pattern
      Animal Life Cycle
      Plant Life Cycle
      Fungal Life Cycle
      Protistan Life Cycles
      Summary

Advantages and Costs of a Sexual Life Cycle
Advantages of a sexual life cycle:
      Diversity by genetic recombination, avoids bad mutation accumulation.
      (implies need for diversity, implies variable environment?)

Costs:
      If environment is stable, might want to preserve genetic combinations?
      Fig. 46.3, handicap of sex
          so if good combination can spread genes in conserved combinations
      Asexual reproduction produces faster population growth...
      See this in some animals, hydra can reproduce sexually or asexually via budding...
          Fig. 13.2 Hydra
(Contrast hydra, where the "bud" does come off, to grass plants were all connected?)

Common Pattern
We are considering eukaryotic options here... prokaryotic "sex" is very different...
Also note that we will cover steriotypes, exceptions to all of these exist...
      Fig. 13.6, sexual life cycles
      eukaryotic life cycles, this figure shows just a few of many possible variants
      Pattern in all sexual life cycles: 2N, zygote, meiosis, 1N, gametes, fertilization
          1N; haploid, one full set of the genome, (i.e. one of each type of chromosome...)
          2N; diploid, two full sets of the genome,
               (i.e. two of each type of chromosome...)
          Meiosis: converts from diploid (2N) to haploid (1N)
          Fertilization: Fusion of two 1N cells to form one 2N cell,
               uses gametes, makes zygote
          zygotes are always single-celled and 2N, may do meiosis or mitosis depending...
          gametes are always single-celled and 1N, do fertilization but no cell division

Optional items:
> whether to do any mitosis at all...
> mitosis allows production of a multicellular organism, not all species do this..
> > or can be used to asexually reproduce...
> whether mitosis is done in 1N side of life cycle and/or 2N side of life cycle
> whether meiosis makes gametes (as in animals), or spores (plants and fungi)...

Animal Life Cycle

Fig. 13.5, human life cycle

As go through life cycle review again... note the "generation" is the multicelled stage

genome	all the genes, on the types of chromosomes that hold them
diploid (2N)	two complete genomic sets in the nucleus
haploid (1N)	one complete genomic set in the nucleus
meiosis	a type of reductive cell division, 2N becomes 1N
mitosis	a type of conservative cell division, keeps same ploidy
gametes	single celled, haploid nucleus, these will do fertilization
zygote	single celled, diploid nucleus, in animals will do mitosis
fertilization	fusing cells, then fusing nuclei so that two-1N nuclei becomes one-2N nucleus, also called "syngamy" two stages of it are: plasmogamy, karyogamy

Note: being mainly diploid, many poor versions of genes (alleles) can be retained....
> if haploid then just one version of each gene, it had better be a good one...
> so there are consequences of being diploid on mutation load carried in genome.

Plant Life Cycle

Fig. 13.6, animal, algal, plant life cycle

Note locations in cycle of

Spore	single celled, haploid, made by meiosis, will not do fertilization, will do mitosis
Gametophyte	Multicellular haploid stage, a "generation" A gamete-producing plant
Gametes	single celled, made by mitosis, not by meiosis, will do fertilization
Sporophyte	Multicellular diploid stage, a "generation" A spore-producing plant

This type of life cycle is called an "alternation of generations"
> since there are two of them..

Fig. 29.13, fern life cycle   Ferns as an example

Notice that mitosis is done by 2N and by 1N stages. So growth and development for both

Note: having a multicellular haploid stage, implies better screening out of poor versions
> of genes than would be seen in animals? An advantage? Can disperse 1N items...

Consider spore production here: Making spores makes cells that become new individuals

  This is involves meiosis, so not asexual reproduction, but is sexual reproduction

  So in plants have sexual reproduction in two places:

    production of spores and zygotes

In both animal and plant life cycles meiosis and the recombining of gametes makes new

  genetic combinations.

  This produces diversity amongst individuals in a population

  The reason for the different multicellular stages are more historic, based on ancestoral

    states, rather than functional...?   Animal life cycle is not the best...

Fungal Life Cycle

  Fig. 13.6, animal, algal, plant life cycle

  (note error in figure from text: fig. 13.6c, no N+N stage?)

  Fig. 31.5, fungal life cycle

  Multicellular haploid stage = hyphae that fold into mycelia

    can live this way for a long time...

  No multicellular diploid stage at all

    only the zygote is 2N in most fungi, this is single-celled.

    zygote immediately does meiosis and makes spores, which are 1N

    So like plants have meiosis-products part of sexual reproduction

      distinct from fertilization part of sexual reproduction

  But fungi also do something weird to their fertilization, divide in up in time...

  Fertilization (syngamy) has two sub-stages

    plasmogamy; fusion of cytosol

    karyogamy; fusion of nuclei

  Fungi have dikaryotic multicellular stage

    (N+N) heterokaryotic the result of plasmogamy

      so a modification of "pace" of fertilization?

      and insertion of cell division into the middle of it?

    this (N+N) body makes up most of what we call a mushroom

    can also form hyphae and mycelia

    can spend decades in this state

  Later in life cycle the nuclei fuse, karyogamy

    to make a diploid zygote

  Example: mushroom life cycle

    Fig. 31.19, life cycle of mushroom

  Note in figure locations of: N+N stage (dikaryotic), zygote, karyogamy and plasmogamy

    and note spores used for dispersal item and made by meiosis

  The N+N state makes the cell functionally diploid,

      even though the nuclei are each haploid

    so since neither truly diploid nor haploid we have this new type of ploidy...

Protistan Life Cycles

life cycles vary all over the place in protists.... here are just two...

Chlamydomonas is a green algae.

> Fig. 28.31, *Chlamydomonas* life cycle
> No multicelled stage here at all. (this is also not covered by fig. 13.6...)
>> so not all eukaryotes have multicelled stage(s)
>> then 1N stage expands population by mitosis, so asexual reproduction
>> note the gametes can be made via meiosis or mitosis...
>> In some cases the cells made by meiosis do mitosis, so are those spores?
>>> could argue that here meiosis makes both spores and gametes??
>> note zygote can go dormant until conditions are good, then does meiosis...
>> Only does sex when conditions become poor "when stressed, think of sex..."

Paramecium sp. (this is not covered in fig. 13.6 either...)

> Fig. 28.11, ciliate life cycle
> Note fusion of cytosols, plasmogamy, preceeds meiosis!
> Note the micronuclei and macronucleus
>> diploid micronucleus, does meiosis
>> creates four haploid micronuclei
>> three degenerate, remaining one divides
>> so in this case start fertilization, then do meiosis and a mitosis in the middle of it!
> This gives a cell with two haploid nuclei... like fungi?
>> Note that syngamy is another name for fertilization
> With cytosol fused (plasmogamy) and swap nuclei
>> this is odd... two cells exchange genetic material and make two cells
>> contrast with more typical fertilization where two gametes make one zygote...?
> then nuclei fuse (karyogamy) forms diploid zygote in each cell, fertilization done.
> The diploid nucleus then divides by mitosis to make 8 diploid nuclei
> four become macronucleus, where genes are expressed
>> cell has four 2N nuclei and one 8N nucleus!!
>> can duplicate further, leading to 50N in macronucleus!
> can then reproduce asexually...
> So still get genetic recombination, but not in what we would see in animals...

Summary

There are many other variations in eukaryotic life cycles... have only covered a few options...
So pattern in all ekaryotic life cycles is

> diploid, meiosis, haploid, fertilization
> All the sexual eukaryotic life cycles must have gametes and zygotes

What varies in eukaryotic life cycles are
  how many multicellular stages and where?
  when is mitosis and meiosis are done? how the gametes are made?
  dikaryotic stage present or not? Spores or not?
  aspects of fertilization done all at once, or divided over time with other stages inserted?

  Other variations exist... in eukaryotes
      and prokaryotes have their own ways of getting new genetic combinations...

Objectives:

What is the difference between haploid and diploid? What other types of ploidy might be possible in eukaryotes, and what types are unlikely to occur in sexual life cycles? What processes are used by eukaryotes to alter their ploidy during their life cycles? Be able to consider the advantages and disadvantages of sexual versus asexual life cycles.

Be able to distinguish and describe the differences between the life cycles of a typical: animal, plant, fungus, and single-celled green alga. In each case what process produces a zygote? In each case what process produces a gamete? In each what is formed as a result of meiosis? Be able to identify any "generation" in the life cycle as well as the presence of any spores and/or dikaryotic stage.

Fertilization (i.e. syngamy) is made up of stages called plasmogamy and karyogamy. What happens in each of these stages and how do these two stages differ?

Which stages of a fungal life cycle can be considered to be haploid, diploid, or dikaryotic? How is a ciliate protist, before it has completed fertilization, similar to that of a dikaryotic fungal cell?

Note: When you look at figures 28.22, 29.13 and 31.19 do NOT worry about the detailed names of specific structures given in these figures. Do worry about being able to locate in a similar figure such items as: Meiosis, mitosis, gametes, any spores, the zygote, and the stages of fertilization. Also be able to describe the ploidy (1N, 2N, and any N+N) of the various stages in these life cycles.

Needed overheads and items:

Fig. 46.3, handicap of sex
Fig. 13.2, Hydra
Fig. 13.6, sexual life cycles
Fig. 13.5, human life cycle
Fig. 13.6, sexual life cycles
Fig. 29.13, fern life cycle
Fig. 13.6, sexual life cycles
Fig. 31.5, fungal life cycle
Fig. 31.19, life cycle of mushroom
Fig. 28.22, *Chlamydomonas* life cycle
Fig. 28.11, ciliate life cycle

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky,
    R.B.Jackson- 2008-<u>Biology</u>. Eighth edition. Pgs. 248-253, 638-640, 812-813,
    997-1000, and figures 13.5, 13.6, 28.11, 28.22, 29.5, 29.13, 31.5, 31.19.
    Pearson/Benjamin Cummings Press. San Francisco, CA.

Related issues:

A sexual life cycle has certain **evolutionary consequences**. Here are some sources that consider a few of these.

Corner E.J.H- 1964-Reproduction and wastage. Chapter 6 in <u>The Life of Plants</u>. University of Chicago Press, Chicago. Pgs. 79-102.

Hedrick P.W- 2000-Selection: Other models and estimates- Chapter 4, pgs. 129-178, in <u>Genetics of Populations</u>- 2nd edition, Jones and Bartlett Publishers. Sudbury, MA.

Zimmer C- 2009-On the origin of sexual reproduction- Science 324: (#5932, 6/5) 1254-1256

In many animal species the life cycle is not the classical one, instead it is a **haplo-diploid life cycle**, in which the males are halpoid and the females are diploid. Here are a few articles that describe some of these species.

Beukeboom L.W., A. Kamping, M. Louter, I.P. Pijnacker, V. Katju, P.M. Ferree, J.H. Werren- 2007-Haploid females in the parasite wasp *Nasonia vitripennis*- Science 315: (1/12) 206

Liu S-S., P.J. DeBarro, J. Xu, J-B. Luan, L-S. Zang, Y-M. Ruan, F-H. Wan- 2007-Asymmetric mating interactions drive widespread invasion and displacement in a whitefly- Science 318: (#5857, 12/14) 1769-1772

Verhulst E.C., L.W. Beukeboom, L. van de Zande- 2010-Maternal control of haplodiploid sex determination in the wasp *Nasonia*- Science 328: (#5978, 4/30) 620-623

Whitfield J- 2007-Who's the queen? Ask the genes- Science 318: (#5852, 11/9) 910-911

Of course, not all members of an animal species actively reproduces. This is particularly seen in the social insects, and there are instances of "helper" **non-reproducing individuals** in other species of animals.

Russell A.F., N.E. Longmore, A. Cockburn, L.B. Astheimer, R.M. Kilner- 2007-Reduced egg investment can conceal helper effects in cooperatively breeding birds- Science 317: (#5840, 8/17) 941-944

Toth A.L., K. Varala, T.C. Newman, F.E. Miguez, S.K. Hutchison, D.A. Willoughby, J.F. Simons, M. Egholm, J.H. Hunt, M.E. Hudson, G.E. Robinson- 2007-Wasp gene expression supports an evolutionary link between maternal behavior and eusociality- Science 318: (#5849, 10/19) 441-444

Other animal species have a form of **asexual life cycle**, involving **parthenogenesis**.

Hayashi Y., N. Lo, H. Miyata, O. Kitade- 2007-Sex-linked genetic influence on caste determination in a termite- Science 318: (#5852, 11/9) 985-987

Meselson M., D.M. Welch- 2007-Stable heterozygosity?- Science 318: (#5848, 10/12) 202-203

Wilson C.G., P.W. Sherman- 2010-Anciently asexual Bdelloid rotifers escape lethal fungal parasites by drying up and blowing away- Science 327: (#5965, 1/29) 574-576

For descriptions of the life cycles of various **protists** see the following:

Ashworth J.M., J. Dee- 1975-The biology of slime moulds. Bulter and Tanner ltd. London, UK.

Burkholder J.M- 1999-The lurking perils of *Pfiesteria*- Scientific American 281: (#2) 42-49

Corner E.J.H- 1964-Living Seaweeds. Chapter 5 in The Life of Plants. University of Chicago Press, Chicago. Pgs. 62-78.

Poxietner M.K., M.L. Carpenter, J.J. Mancuso, C-J.R. Wang, S.C. Dawson, W.Z. Cande- 2008-Evidence for karyogamy and exchange of genetic material in the binucleate intestinal parasite *Giardia intestinalis*- Science 319: (#5869, 3/14) 1530-1533

Raven P.H., R.F. Evert, S.E. Eichhorn- 1992-Protista II: Red, brown, and green algae. Chapter 14, pgs. 268-297. In, Biology of Plants. Fifth edition. Worth Publishers. New York, New York.

Saunders G.W., M.H. Hommersand- 2004-Assessing red alga supraordinal diversity and taxonomy in the context of contemporary systematic data- American Journal of Botany 91: (10) 1494-1507

For a description of the **plant** life cycle and its likely origins see:

Graham L.E- 1985-The origin of the life cycle of land plants- American Scientist- 73: 178-186

Kaplan D.R., T.J. Cooke- 1996-The genius of Wilhelm Hofmeister: The origin of causal-analytical research in plant development- American Journal of Botany 83: (#12) 1647-1660

Singer S.R- 1997-Plant life cycles and angiosperm development- Chapter 23, pgs 493-513 in Embryology: Constructing the organism. S.F. Gilbert and A.M Raunio editors. Sinauer Assoc., Sunderland, MA.

We are not completely limited by our life cycle. For instance, there are **haploid human cell lines** that have been established and are used in research. Here is an example.

Carette J.E., C.P. Guimaraes, M. Varadarajan, A.S. Park, I. Wuethrich, A. Godarova, M. Kotecki, B.H. Cochran, E. Spooner, H.L. Ploegh, T.R. Brummelkamp- 2009- Haploid genetic screens in human cells identity host factors used by pathogens- Science 326: (#5957, 11/27) 1231-1235

BIO 108   2010
Day 1, Lecture 2, Title: Cell Division.
Text Readings: Campbell et al. (2008) Chapter 12.

Topics to cover:

> **Eukaryotic Cell Cycle**
> > **Ploidy and Chromosome Issues**
> > **Cell Cycle Controls**
> > **Mitosis**
> > **Cytokinesis**
> **Cell Division Variants**
> **Prokaryotic Binary Fission**
> **Principle of Segregation**

Eukaryotic Cell Cycle
> (Warning: deceptive topic, everyone thinks they know cell division and mitosis...)
Cell cycle
> Note Replication differs from Reproduction
> > Replication is molecular, makes new molecules, not new individuals
> > Reproduction is cellular/organismal; makes new cells and perhaps new individuals
> > > cell division is often needed for organism growth
> > Fig. 12.5, cell cycle
> M phase, has two parts: period of cell division, might be mitosis or meiosis
> > mitosis   just separation of genetic material into two nuclei
> > cytokinesis   separation of the cytosol and the two nuclei
> > > this is when reproduction may occur, sexual or asexual
> > (Many people when they refer to "mitosis" really mean M phase
> > > as they include cytokinesis in their thinking...)
> G1 and G2   "gaps" periods of growth, and organellar production
> > During G2 the centrosomes, with any centrioles, are replicated.
> S   time of most nuclear DNA replication
> > > so get sister chromatids in each chromosomes this way
> > Note DNA in organelles can replicate here and at other times...

> **Ploidy and Chromosome Issues**
Changes in chromosome structure over cell cycle
> > Fig. 16.21, chromosome structure and condensation
> chromatin is protein and DNA, note changes in condensation across cell cycle
> > Fig. 12.4, chromosome SEM and sister chromatids
> > (note: it does not duplicate while condensed)
> Have to have double chromatid chromosomes at start of normal M-phase
> > note centromere, chromatids, DNAs

Normally have single chromatid chromosome at end of cell division
    consider number of DNA molecules, one dsDNA per chromatid
DNA replication during S phase is then done to get ready again for cell division
Note: Mitosis can be done by a haploid or by a diploid cell
    (Ask students to work through examples of diploid or haploid cell mitosis)

Confusing doubles, note the hierarchy of level of organization over which they occur:
    double-stranded DNA   molecular level
    Double-chromatid chromosome   multi-molecular, DNA and proteins
    Homologous pair   two similar chromosomes
    Diploid (2N)   two sets of the genome
Draw out on board chromosome, haploid/diploid states
    Show changes with:
            Mitosis vs. meiosis
            double vs. single chromatid chromosomes
            DNA replication
            meiosis
            fertilization

Cell Cycle Controls
Need to slow/halt cell cycle to
        replicate molecules or make more organelles, repair damaged DNA sections...
        wait for external signals to trigger entry into M phase, etc...
One system of control involves proteins called cyclins, many types of these exist
        Fig. 12.17, cell cycle controls, cyclins
Across cell cycle, note changes in concentrations of
        cyclin
        cyclin-dependent kinase
Fig. 12.13, cell fusion experiment
        fuse to form M-phase promoting factor (MPF)
        if inject MPF into a cell can induce M phase
errors in cell cycle controls are associated with cancers

Mitosis
Note that this is just one part of M-phase.
        Mitosis is the means to get two nuclei formed from one nucleus.
Will present a sterotype here, variations and exceptions exist
        Fig. 12.6, mitosis stages
        (Note: can stain chromatin vs. stain of microtubules vs. stain of microfilaments)
Phases of mitosis: PPMAT
        prophase, prometaphase, metaphase, anaphase, telophase
Fig. 12.6a, (Campbell and Reece, 2005) mitosis

Prophase
>	condensation of chromosomes
>>	critical as each dsDNA molecule is 2-6 cm long uncondensed
>>	condensed fully it gets down to about 1-2 µm long
>>	do this by wrapping DNA up with proteins,
>>	the complex of DNA and proteins is called chromatin
>>	This condensation can interfere with replication and gene expression...
>	centrosomes, with any centrioles (mostly animals but also others), move to poles
>	spindle (made of microtubules) begins to form

Prometaphase
>	rupture of nuclear membrane into many small vesicles
>>	will be used later to form new nuclear membranes
>	spindle enters former nuclear space
>>	connects to kinetochores on chromosomes, at centromeres
>>	kinetochore: region of centromere where microtubules attach
>>>	need two kinetochores per centromere
>>	Fig. 12.7, spindle and kinetochores
>	chromosomes begin to be moved towards common plate

Metaphase
>	Fig. 12.6b, (Campbell and Reece, 2005) mitosis
>	chromosomes at metaphase plate
>	in this figure note: two sets of chromosomes, 2N = 4 (text has 2N = ? ... what?)
>	a cell cycle checkpoint here,
>>	uncovered kinetochores are detected and inhibit progress to anaphase

Anaphase
>	once all kinetochores are attached to microtubules then
>>	with a calcium ion spike get separation of chromatids
>	each double chromatid chromosome becomes two single chromatid chromosomes
>	now 2X as many chromosomes in the entire cell
>>	What happened to the ploidy? How many genetic sets in the cell?
>>	now 8 chromosomes, if 2N = 4, 8 chromosomes = 4N!
>>	two 2N sets are being pulled apart
>	chromosomes segregated as full genomic sets
>>	Segregation is separation of items
>>	what is being segregated during anaphase? (full sets of the genome)
>>	note right now there is NO separation of other organelles...
>>>	that happens in cytokinesis
>>	in this figure, 2N moving up, 2N moving down, in full sets
>>>	consider how this would be different if a haploid cell did mitosis?
>>	it would make problems to separate by DNA mass if do not get sets right...

Telophase
>   migration of single-chromatid chromosomes stops
>   nuclear envelop reforms from vesicles from original and new material
>   chromosomes decondense
>   This forms a cell with two nuclei, not two cells

Cytokinesis
Cytokinesis is NOT part of mitosis, but it is part of M phase
Notice: mitosis makes two nuclei from one, NOT two cells.
Mitosis alone would result in two nuclei per cell
separation of cytosol
>   need careful division of the cytosolic contents
>   mitochondria, chloroplasts, centrosomes....
>   Fig. 12.9, cytokinesis options

animal/plant differences
>   contrast cleavage furrow versus cell plate mechanisms
>   animal cells use microfilaments and motor proteins to pinch off
>   plant cells fuse vesicles along the cell plate to make new membrane and cell wall

Cell Division Variants
Variations on Mitosis, the above was a steriotype, now consider a few variations
Fig. 12.12, variations in mitosis
>   Nuclear membrane need not degrade
>   spindle may form inside nuclear space rather than in cystosol
>   chromosomes may attach to inner membrane surface of nucleus
>   this allows cytosolic spindle to pull nuclear chromosomes through membrane
Obviously, the cytokinesis need not be equal, daughter cells need not be equal in size

Prokaryotic Binary Fission
Binary Fission is a type of cell division seen in prokaryotes
Fig. 12.11, binary fission
>   Two DNA molecules separated (i.e. segregation of genetic material)
>   A tubulin-like protein involved in connecting DNA to membrane
>   and in pulling the DNA molecules apart
>   This is like the start of anaphase of mitosis?
This is also the pattern of division use by chloroplasts and mitochondria in eukaryotes

4

Principle of Segregation

       Fig. 12.6b, (Campbell and Reece, 2005) mitosis stages

This is the classical Mendelian principle

       genes of a species are spread across the chromosomes of that species

       number of chromosomes used varies amongst species

       Need to segregate/separate the genes properly as full genetic sets into daughter cells

Mitosis: results in nuclei with proper genomic sets and conserves original ploidy

Cytokinesis: process of separation of cytosol and the two nuclei so that

       each of the two daughter cells gets needed organelles and other items

       Both involve separation, one does genetic sets, other does cell contents....

Cell division allows for growth of a multicelled organism,

       can also allow for asexual reproduction in some cases.

       Obviously it is much more complex than we have covered here....

(If time: show movies 18.1, 18.2, 18.4)

Objectives:

Be able to describe how cyclin and cyclin-dependent kinases are used to control the transition between various stages of the cell cycle.

Know the stages of mitosis (and meiosis, which will be covered in a subsequent lecture). Fig. 12.6 in the text is a useful summary of events, but be able to generalize it to other species that have a different number of types of chromosomes than are shown in this figure. What event(s) are associated with the start and completion of each stage?

Both mitosis and meiosis involve the process of segregation. During mitosis what is being segregated and why is this critical? Mitosis is said to be a conservative type of cell division while meiosis is a reductive type of cell division; what is being conserved and what is being reduced in each of these cases?

How do chromosomes at the start of cell division differ from chromosomes at the end of cell division? What are homologous chromosomes, what makes them homologous? What are sister chromatids and are they each considered to be a separate chromosome? Does decondensation of single-chromatid chromosomes, or their replication after M-phase, alter the ploidy of a cell? How does the total number of chromosomes, and the ploidy, in a cell change as it undergoes mitosis (or meiosis)? What is a kinetochore, where on a chromosome does it occur, and what role does it play?

How does mitosis differ from cytokinesis? What structures are often used in a eukaryotic cell to achieve cytokinesis, and are these in any way similar to the structures used during segregation? What is the result if mitosis occurs without cytokinesis right after it? Must the nuclear envelope always break down during mitosis?

What occurs during binary fission in bacteria? For each event you identify ask yourself which phase of mitosis or M-phase of the eukaryotic cycle is most similar to it.

For review see self-quiz questions #3, 7, 9 and 10 at the end of chapter 12.

Needed overheads and items:

       Fig. 12.5, cell cycle
       Fig. 16.21, chromosome structure and condensation
       Fig. 12.4, chromosome SEM and sister chromatids
       Fig. 12.17, cell cycle controls, cyclins
       Fig. 12.13, cell fusion experiment
       Fig. 12.6, mitosis stages
       Fig. 12.6a, (Campbell and Reece, 2005) mitosis
       Fig. 12.7, spindle and kinetochores
       Fig. 12.6b, (Campbell and Reece, 2005) mitosis
       Fig. 12.9, cytokinesis options
       Fig. 12.12, variations in mitosis
       Fig. 12.11, binary fission
       Fig. 12.6b, (Campbell and Reece, 2005) mitosis

Also available (if time):
       quicktime movies from MBOTC CD
       18.1
       18.2
       18.4

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-The cell cycle. Chapter 12. Pages 228-245. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition, Figure 12.6. Benjamin Cummings Press. San Francisco, CA.

Cell Biology, Interactive CD. For, <u>Molecular Biology of the Cell</u>. 4th edition. Movies 18.1, 18.2, 18.4. Garland Science Publishing. N.Y., N.Y.

Related issues:

Here are some reviews of **mitotic cell division** and some of its variations.

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walker- 2002-The mechanics of cell division. Chapter 18, pgs. 1027-1062, in <u>Molecular Biology of the Cell</u>. 4th edition. Garland Science, N.Y., N.Y.

Pickett-Heaps J.D., B.E.S. Gunning, R.C. Brown, B.E. Lemmon, A.L. Cleary- 1999-The cytoplast concept in dividing plant cells: Cytoplasmic domains and the evolution of spatially organized cell division- American Journal of Botany 86: (#2) 153-172

The role of other items than the microtubules in the **spindle apparatus** has lead to a proposal of a **spindle matrix** with many other items present than just the microtubules.

Caudron M., G. Bunt, P. Bastiaens, E. Karsenti- 2005-Spatial coordination of spindle assembly by chromosome-mediated signaling gradients- Science 309: (8/26) 1373-1376

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Goshima G., R. Wollman, S.S. Goodwin, N. Zhang, J.M. Scholey, R.D. Vale, N. Stuurman- 2007-Genes required for mitotic spindle assembly in *Drosophila* S2 cells- Science 316: (4/20) 417-421

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Travis J- 2007-Return of the matrix- Science 318: (#5855, 11/30) 1400-1401

For more on **segregation** of chromosomes and the interaction of **sister chromatids** during mitosis see:

Folco H.D., A.L. Pidoux, T. Urano, R.C. Allshire- 2008-Heterochromatin and RNAi are required to establish CENP-A chromatin at centromeres- Science 319: (#5859, 1/4) 94-97

Hutchins J.R.A., Y. Toyoda, B. Hegemann, I. Poser, J-K. Hériché, M.M. Sykora, M. Augsburg, O. Hudecz, B.A. Bushhorn, J. Bulkescher, C. Conrad, D. Comartin, A. Schleiffer, M. Sarov, A. Pozniakovsky, M.M. Slabicki, S. Schloissnig, I. Steinmacher, M. Leuschner, A. Ssykor, S. Lawo, L. Pelletier, H. Stark, K. Nasmyth, J. Ellenberg, R. Durbin, F. Buchholz, K. Mechtler, A.A. Hyman, J-M. Peters- 2010- Systematic analysis of human protein complexes identifies chromosome segregation proteins- Science 328: (#5970, 4/30) 593-599

Javerzat J-P- 2010-Directing the centromere guardian- Science 327: (#5962, 1/8) 150-151

Kawashima S.A., Y. Yamagishi, T. Honda, K-i. Ishiguro, Y. Watanabe- 2010- Phosphorylation of H2A by Bub1 prevents chromosomal instability through localizing Shugoshin- Science 327: (#5962, 1/8) 172-177

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Pesin J.A., T.L. Orr-Weaver- 2008-Regulation of APC/C activators in mitosis and meiosis- Annual Review of Cell and Developmental Biology 24: 475-499

Rosasco-Nitcher S.E., W. Lan, S. Khorasanizadeh, P.T. Stukenberg- 2008-Centromeric aurora-β activation requires TD-60, microtubules, and substrate priming phosphorylation- Science 319: (#5862, 1/25) 469-472

Ström L., C. Karlsson, H.B. Lindroos, S. Wedahl, Y. Katou, K. Shirahige, C. Sjögren- 2007-Post-replicative formation of cohesion is required for repair and induced by a single DNA break- Science 317: (#5835, 7/13) 242-245

Ünal E., J.M. Heidinger-Pauli, D. Koshland- 2007-DNA double-strand breaks trigger genome-wide sister-chromatid cohesion through Eco1 (Ctf7)- Science 317: (#5835, 7/13) 245-248

Watrin E., J-M. Peters- 2007-How and when the genome sticks together- Science 317: (#5835, 7/13) 209-210

Here is an article that examines the **segregation** of plasmids and cytosolic structures, such as carboxysomes in which carbon fixation is done, in bacteria during **binary fission**.

Garner E.C., C.S. Campbell, D.B. Welbel, R.D. Mullins- 2007-Reconstitution of DNA segregation driven by assembly of a prokaryotic actin homolog- Science 315: (3/2) 1270-1274

Savage D.F., B. Afonso, A.H. Chen, P.A. Silver- 2010- Spatially ordered dynamics of the bacterial carbon fixation machinery- Science 327: (#5970, 3/5) 1258- 1261

During normal segregation the two sister chromatids are equivalent. Here are articles that examine a situation in which the sister chromatids are not equivalent, so that there is **chromatid assortment** of the different versions during mitosis.

Armakolas A., A.J.S. Klar- 2007-Left-right dynein motor implicated in selective chromatid segregation in mouse cells- Science 315: (1/5) 100-101

Sapienza C- 2007-Do Watson and Crick motor from X to Z?- Science 315: (1/5) 46-47

How forces are generated during **cytokinesis** is of much interest, as well as how cytokinesis occurs in various species and the items it must separate.

Canman J.C., L. Lewellyn, K. Laband, S.J. Smerdon, A. Desai, B. Bowerman, K. Oegama- 2008-Inhibition of Ras by the GAP activity of central spindlin is essential for cytokinesis- Science 322: (#5907, 12/5) 1543-1546

Carlton J.G., J. Martin-Serrano- 2007-Parallels between cytokinesis and retroviral budding: A role of the ESCRT machinery- Science 316: (#5833, 6/29) 1908-1912

Cook M.E- 2004-Cytokinesis in *Coleochaete orbicularis* (Charophyceae): An ancestral mechanism inherited by plants- American Journal of Botany 91: (#3) 313-320

Knoblich J.A- 2006-Sara splits the signal- Science 314: (11/17) 1094-1096

McIntosh K., J.D. Pickett-Heaps, B.E.S. Gunning- 1995-Cytokinesis in *Spirogyra*: Integration of cleavage and cell-plate formation- International Journal of Plant Science 156: (#1) 1-8

Vavylonis D., J-Q. Wu, S. Hao, B. O'Shaughnessy, T.D. Pollard- 2008-Assembly mechanism of the contractile ring for cytokinesis by fission yeast- Science 319: (#5859, 1/4) 97-100

The **cell cycle** is complex and tightly regulated. Here are some articles studying aspects of it.

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Francis D., N.G. Halford- 1995-The plant cell cycle- Physiologica Plantarum 93: 365-374

Hemerly A.S., S.G. Prasanth, K. Siddiqui, B. Stillman- 2009-Orc1 controls centriole and centrosome copy number in human cells- Science 323: (#5915, 2/6) 789-793

Jacobs T.W- 1995-Cell cycle control- Annual Review of Plant Physiology and Plant Molecular Biology 46: 317-339

Kodama H., M. Ito, A. Komamine- 1994-Studies of the plant cell cycle in synchronous cultures of *Catharanthus roseus* cells- Plant and Cell Physiology 35: (#4) 529-537

Müller H., M-L., Fogeron, V. Lehmann, H. Lehrach, B.M.H. Lange- 2006-A centrosome-independent role for γ-TuRC proteins in the spindle asembly checkpoint- Science 314: (10-27) 654-657

Torres-Rosell J., G. DePiccoli, V. Cordon-Precidado, S. Farmer, A. Jarmuz, F. Machin, P. Pasero, M. Lisby, J.E. Haber, L. Aragón- 2007-Anaphase onset before complete DNA replication with intact checkpoint reponses- Science 315: (3/9) 1411-1415

Weinert T- 2007-What a cell should know (But may not)- Science 315: (3/9) 1374-1375

Yang Y., A.H. Kim, T. Yamada, B. Wu, P.M. Bilimoria, Y. Ikeuchi, N. de la Iglesia, J. Shen, A. Bonni- 2009-A Cdc20-APC ubiquitin signaling pathway regulates presynaptic development- Science 326: (#5952, 10/23) 575-578

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Miyagishima S., R. Itoh, K. Toda, H. Kuroiwa, T. Kuroiwa- 1999-Real-time analysis of chloroplast and mitochondrial division and differences in the behavior of their dividing rings during contraction- Planta 207: 343-353

Osteryoung K.W- 2000-Organelle fission. Crossing the evolutionary divide- Plant Physiology 123: 1213-1216

Pyke K.A., A.M. Page- 1998-Plastid ontogeny during petal development in *Arabidopsis*- Plant Physiology 116: 797-803

Robertson E.J., S.M. Rutherford, R.M. Leech- 1996-Characterization of chloroplast division using the *Arabidopsis* mutant *arc5*- Plant Physiology 112: 149-159

White D- 2000-Growth and cell division- Chapter 2, pgs. 37-65, in, The Physiology and Biochemistry of Prokaryotes. 2nd edition. Oxford University Press, N.Y.

Yoshida Y., H. Kuroiwa, O. Misumi, K.Nishida, F. Yagisawa, T. Fujiwara, H. Nanamiya, F. Kawamura, T. Kuroiwa- 2006-Isolated chloroplast division machinery can actively constrict after stretching- Science 313: (9/8) 1435-1438

BIO 108   2010
Day 1, Lecture 3, Title: Mendelian Genetics and Meiosis.
Text Readings: Campbell et al. (2008), chap. 13, and pgs. 262-271.

Topics to cover:

> **Review Chromosome States in Life Cycle**
> **Meiosis**
> **Mendelian Genetics**
> > **What he did**
> > **What new concepts he developed**
> **Representing Genotypes on Chromosomes**
> **Punnett Square**

Review Chromosome States in Life Cycle
Use drawings on board of chromosomal state across life cycle
> Show changes with:
> > Mitosis vs. meiosis
> > double vs. single chromatid chromosomes
> > DNA replication
> > meiosis
> > fertilization
> This links cell division steps to life cycle events
> > Fig. 13.9, figure and table, mitosis vs. meiosis,   note misleading aspects of figure,
> > 1. can be 1N OR 2N and do mitosis, also replication not typically done when
> > > chromosomes are condensed as they are during cell division...
> > 2. can have crossing over in 2N cell of mitosis, just very rare..
> > > Crossing over occurs during almost every meiotic division
> > > Crossing over occurs about once-in-a-million of mitotic divisions
> > 3. Also, meiosis can make either gametes or spores, depending on the life cycle.
> > > In plants, meiosis makes spores. In animals, meiosis makes gametes.
> > 4. Sister chromatids exist in a single double chromatid chromosome, at the start of
> > > anaphase they become distince single chromatid chromosomes which are then
> > > separated. So from anaphase on there are no sister chromatids anymore...

Meiosis
Stages of Meiosis: a reductive cell division, alters ploidy
> > Fig. 13.8, stages of meiosis
> > focus on ploidy of cell, 2N to 1N to 2N to 1N after cytokinesis
> > focus on ploidy of groups of chromosomes 2N to 1N
> > Note how homologous pairs of double-chromatid chromosomes
> > > separate in anaphase I
> > and how homologous pairs of single-chromatid chromosomes separate at anaphase II

> Prophase I, see Synapsis

get four chromatids in a homologous pair, these four are called a "tetrad"
>Four DNA molecules present, so can send one DNA molecule to each of
>>the four nuclei that will be formed by meiosis
>typically have crossing over between parts of homologous chromosomes
>>this mixes genetic material in the DNA molecules...

Segregation, separation of complete genomic sets
>first division segregation, during anaphase I
>second division segregation, during anaphase II
>>notice that here get separation of versions of genes, alleles
>>thus haploid cell produced will have just one genetic version per gene
>>Given that crossing over has occurred the two chromatids per chromosome
>>>are not equivalent, so need second division separation of sets
>>In fig. 13.8, anaphase II: Text refers to "sister chromatids", I disagree..
>>Note that what is being separated are pairs of homologous chromosomes.
>>>I would not call them sister chromatids, as chromatids are parts of
>>>>ONE chromosome... Here we have distinct chromosomes.
>(Recall that there is segregation also during mitosis and binary fission...)
>Will come back to this...

Changes in Ploidy
>Fig. 13.7, meiosis overview
>note: Replication is in S-phase of cell cycle, during interphase
>>So top of the figure is the interphase before Meiosis
>>>but chromosomes are not typically condensed then...
>>Also at step 2, chromosomes are being separated, not chromatids.
>>>Chromatids are parts of chromosomes....
>A 2N cell, at top of figure, through meiosis, used to make four 1N cells at bottom

Independent assortment
>Fig. 13.11, independent assortment
>first division independent assortment
>Note this suggests that maternal and paternal chromosomes stay distinct
>>assumes no crossing over. This is possible, but unusually rare...
>Fig. 13.12, crossing over
>Note with crossing over the products of meiosis I are
>>fusions of maternal and paternal genetic material
>>It is in their children's meiosis that the parents genetic material finally fuses.
>Need second division of meiosis to separate the non-similar chromatids
>First division independent assortment, during metaphase I
>>Here indep. assort. between the homologous pairs of chromosomes
>Second division independent assortment, if there was crossing over
>>then there is assortment during metaphase II.
>>>Here what is independent is how the double-chromatid chromosomes
>>>>assort relative to each other

Mendelian Studies and Genetics

Mendelian studies
      Fig. 14.00x, (Campbell and Reece, 2002) Mendel
      Common thinking at his time was some sort of blending inheritance
      At his time many researchers tried to generalize from individual or a few events...

What he did

Things he did that were unusual
      selected distinct/clear traits,
            NOT interested in blended traits
            Table 14.1, traits and outcomes of crosses
      selected true breeding lines,

      able to control breeding, this helps control for possible confounding variables...
            Fig. 14.2, pea breeding system
      did multiple crosses
            NOT just one, that was unusual for his time
      did crosses across several generations
            NOT just one, also a very unusual move for his time
      numerical analysis of results
            this let him generalize
            but not typical amongst biologists of his time...
                 much harder, as has to account for rare but valid events...

What new concepts he developed

Some important items that came from his work
      Particulate genetics: these traits are particulate,
            not blending, but actual distinct particles
            this led to idea of distinct genetic factors; concepts of genes....
      Alternate genetic forms:
            we now call these alleles for the gene
      Dominance/Recessive relations between these forms:
            some are dominant to others
            so recessive traits only seen when homozygous
            This led to notion of genotype distinct from phenotype
                 Fig. 14.6, genotype vs. phenotype
      Test cross:
            he developed the idea of a "test" or "back" cross
                 Fig. 14.7, test cross
      The traits move as sets, which segregate:
            Table 14.1, traits and outcomes of crosses
            He noted that the traits he examined were inherited to next generation
                 For instance, Never lose trait of flower color
            So there had be a genetic factor coding for each of these traits,
                 and these factors had to be passed on as sets

this has led to our concept of ploidy and of the genome

Contrast with how text describes this concept

"... the two alleles for a heritable character separate (segregate) during gamete formation and end up in different gametes." (Campbell et al. 2008; pg. 266)

This is a too restricted a definition of segregation

Problems with it:

Gamete formation: Mitosis can make gametes, depends on life cycle

Segregation does not have to involve any change in ploidy

occurs during mitotic cell division, occurs during binary fission

Independent assortment of genetic factors:

Different traits assort independently

Fig. 14.8, dependent vs. independent assortment

(note how chromosomes would be in each case)

YR/yr implies alleles Y and R are linked in one chromosome and alleles y and r are linked in another homologous chromosome..

this would give dependent assortment, due to linkage

YyRr implies alleles Y and y are on different chromosomes of one homologous pair, while alleles R and r are on different chromosomes of another homologous pair.

This would allow independent assortment to be possible...

Representing Genotypes on Chromosomes

Consider genotype YyRr, how to arrange these alleles on chromosomes

Several possible ways....

Draw out examples with alleles indicated

How to represent genetic state of a cell, and represent alleles on chromosomes

YR/yr versus YyRr; in once case are linked, in other case or not

Homozygous/heterozygous, dominant recessive

So from Mendelian genetics have two principles

see these during meiosis...

segregation

first and second division segregation, during Anaphase I and II

(also occurs during other types of cell division, such as mitosis and binary fission)

Independent assortment

during Metaphase I (and metaphase II if there was crossing over...)

Different items are assorting during these two stages...

Punnett Square

Mendel did not come up with this way of representing crosses, it came later
but is based on Mendelian model

Assume:

Parents   PP X pp
All possible gametes from each parent placed along sides
F1: All possible zygotes shown in square
Do F1 X F1 cross, and note ratio gotten in F2

Monohybrid and Dihybrid crosses (note these are not the only types of crosses. what are others?)

Fig. 14.5, monohybrid cross
assumes classical Mendelian dominant/recessive relationships
gives a 3:1 phenotypic ratio

Proper segregation implies that distinct genetic factors persist as distinct factors
but also move as full sets. This explains why sets of factors can persist.
Blending inheritance would have resulted in loss of white phenotype,
its persistence in the line suggests that factors are distinct...

Fig. 14.8, dihybrid cross
assuming independent assortment of genes involved
and classical dominant/recessive relationships
also see proper segregation of full sets of the genome here
gives a 9:3:3:1 phenotypic ratio
note these ratios are for F2 in hybrid crosses
other ratios will occur for other generations or other types of crosses

Students should read about rules of mutiplication and addition.... from text (section 14.2)....
see selected questions at end of the chapters.

Objectives:

Know the stages of meiosis. You should be able to work though the stages in figure 13.8 and for a given cell at any given stage be able to determine: The ploidy of the cell at that stage. Whether homologous chromosomes are present or not. Consider what sister chromatids are, how they are made, and what they would become when separated. Does the total number of chromosomes and/or DNA molecules change during meiosis? Be able to relate the appropriate stages of meiosis to the Mendelian principles of segregation and independent assortment. At each stage where they occur be able to describe what is being segregated, and between what things there is independent assortment. How are mitosis and meiosis similar? How do they differ? Crossing over is so much more likely during meiosis that it can be assumed to happen every time, what consequences does this have for events that occur during the second division of meiosis?

Be able to describe the Mendelian model of genetics. What were the new concepts that Mendel devised through his studies? Be able to describe the importance of each of his findings to our understanding of genetics. What were some of the ways that Mendel improved his breeding experiments relative to those done by others before him?

Be able to use the Punnett square to model a cross. Be able to identify the possible gametes, and if given a genotype be able to draw a possible arrangement of the alleles involved on chromosomes as they might exist at a give stage of meiosis.

Given a cross be able to calculate the probability of obtaining a given genotype amongst the offspring. Review the uses of the rules of multiplication and of addition in working problems in probabilities.

[Make sure you note the errors in figure 13.9 of the text. Also note that chapter 14 does a very poor job in describing the principles of segregation and of independent assortment. What it gives concerning segregation is often only true for extremely limited conditions, and in too many cases is inaccurate. See the handout on these principles. What I give you there is more consistent with the way cell biologists view these principles, but be aware that you may encounter views like what the text presents when dealing with geneticists.]

For review see self-quiz questions #5, 6, 8 and 9 of chapter 13. (In my view choices (a), (b), (c) and (e) are all valid answers for question #3, do you see why? Do you see how this relates to the principle of segregation?)

Also for review, see self-quiz quesitons #1-4, 9 and 10 of chapter 14.

BIO 108   Lect 3   (Handout)

Mendelian Principles:

Principle of Independent Assortment.
The text proposes the following definition:
"Law of Independent Assortment... each pair of alleles segregates independently of each other pair of alleles during gamete formation." (Campbell et al., 2008: pg. 269)

I have four issues with the way the text defines this Mendelian Principle.

1.) The use of the phrase "segregates independently" is unfortunate as it suggests that segregation and independent assortment are similar processes, when they really are not. This phrase could be replaced with the phrase "assorts independently" with no loss of meaning and perhaps maintain the distinction between segregation and independent assortment? Cell biologists would view "independent segregation" during cell division as something that is seen during non-disjunction.

2.) The phrase "each pair of alleles" might be referred to as "each pair of genetically unlinked alleles" so that those alleles that are genetically linked are clearly excluded since they are known to violate this principle?

3.) The reference to "gamete formation" is unfortunate as plants, fungi, and many other species make their gametes by mitosis during which there is normally no independent assortment. The above definition seems to wish to limit application of this Mendelian principle just to the animal life cycle? So I would suggest dropping the reference to gamete formation entirely.

4.) It might also be pointed out that independent assortment occurs during metaphase I (between homologous pairs of double chromatid chromosomes) and due to crossing over it also often occurs during metaphase II (between double chromatid chromosomes that have differences in their chromatids). The definition in the text makes no mention of when independent assortment takes place.

Therefore, I would suggest the following as a replacement.

Law of Independent Assortment: Each pair of differing alleles assorts independently from other pairs of differing alleles from which it is genetically unlinked during metaphase I of meiosis when double chromatid chromosomes line up at the metaphase I plate. And often there is independent assortment during metaphase II of meiosis between of the double chromatid chromosomes that line up at the metaphase II plate due to the different alleles on their different chromatids as a result of crossing over.

<u>Principle of Segregation</u>.
The text proposes the following definition:
"Law of Segregation... the two alleles for a heritable character segregate (separate) during gamete formation and end up in different gametes." (Campbell et al., 2008: pg 266)

I have three issues with this definition as given by the text.

1.) The references to "gametes" and "gamete formation" are unfortunate as it implies that segregation only occurs during eukaryotic sexual life cycles, and then only at one point in the life cycle. In reality segregation occurs as a part of any successful cell division including meiosis, mitosis, and binary fission. Obviously not all these cell divisions result in gamete formation.

2.) The focus on the level of alleles, while technically correct, is perhaps limited. These alleles need not be different, or from different parents, for segregation of them to occur. To be fully inclusive there should be mention of segregation also at the level of chromosomes to separate homologous chromosomes, and at the level of genomes to separate full sets of the genome. Obviously, failure to achieve segregation results in non-disjunction. So there is a great deal of selective pressure to achieve proper segregation of the genetic material during cell division.

3.) There is no mention made in the text definition of when this process is carried out. That it occurs in anaphase of mitosis, and during anaphase I and II of meiosis should be mentioned. I will just point out that during anaphase of mitosis, and anaphase II of meiosis, the previous double-chromatid chromosomes that were present have become two single-chromatid chromosomes that then represent a homologous pair of chromosomes. These homologous chromosomes must then be separated at anaphase even when a haploid cell is carrying out mitosis. Thus segregation is not dependent on genotype or on ploidy, as full sets of the genome must still be separated. A similar process is done during binary fission when two homologous bacterial chromosomes must be separated.

Therefore I would suggest the following phrasing:

Law of Segregation: At the allele level segregation ensures that pairs of alleles for a heritable character are separated, at the chromosome level it ensures that each chromosome is separated from its homolog, and at the genome level it ensures that full sets of the genome are separated into the resulting daughter cells. This is normally done during anaphase of mitosis, anaphase I and II of meiosis, and during binary fission in prokaryotes.

Needed overheads and items:

Fig. 13.9, figure and table, mitosis vs. meiosis
Fig. 13.8, stages of meiosis
Fig. 13.7, meiosis overview
Fig. 13.11, independent assortment
Fig. 13.12, crossing over
Fig. 14.00x, (Campbell and Reece, 2002) Mendel
Table 14.1, traits and outcomes of crosses
Fig. 14.2, pea breeding system
Fig. 14.6, genotype vs. phenotype
Fig. 14.7, test cross
Fig. 14.8, dependent vs. independent assortment
Fig. 14.5, monhybrid cross
Fig. 14.8, dihybrid cross

Handout:
Mendelian Principles.stm

Bring book?

Corcos A.F., F.V. Monaghan- 1993-Gregor Mendel's Experiments on Plant Hybrids: A guided study. 220 pages. Rutgers University Press. New Brunswick, N.J.

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky,
R.B.Jackson- 2008m-Meiosis and sexual life cycles. Chapter 13. Pages 248-
261, and pgs. 262-271. Biology. Eighth edition. Pearson/Benjamin Cummings
Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-Biology, 6th edition, Fig. 14.00x.. Benjamin Cummings
Press. San Francisco, CA.

Corcos A.F., F.V. Monaghan- 1993-Gregor Mendel's Experiments on Plant Hybrids: A
guided study. 220 pages. Rutgers University Press. New Brunswick, N.J.

Related issues:

What is the view on how **meiosis evolved**?

Cleveland L.R- 1947-The origin and evolution of meiosis- Science 105: 287-288

Mealey L- 2000-Evolution of sex and sex differences. Chapter 3, pgs. 39-68. In, <u>Sex Differences. Developmental and Evolutionary Strategies</u>. Academic Press, San Diego, CA.

Villeneuve A.M., K.J. Hillers- 2001-Whence meiosis?- Cell 106: 647-650

How do **homologous chromosomes** find each other during meiosis? This study looks at aspects of this issue:

Tsubouchi T., G.S. Roeder- 2005-A synaptonemal complex protein promotes homology-independent centromere coupling- Science 308: (5/6) 870-873

Here are some studies of **crossing over** during **meiosis**. It turns out that there are certain "hotspots" with very high cross over frequencies in some areas of the chromosomes.

Baudat F., J. Buard, C. Grey, A. Fledel-Alou, C. Ober, M. Przeworski, G. Coop, R. de Massy- 2010-PRDM9 is a major determinant of meiotic recombination hotspots in humans and mice- Science 327: (#5967, 2/12) 836-840

Cheung V.G., S.L. Sherman, E. Feingold- 2010-Genetic control of hotspots- Science 327: (#5967, 2/12) 791-792

Myers S., R. Bowden, a. Tumian, R.E. Bontrop, C. Freeman, T.S. MacFie, G. McVean, P. Donnelly- 2010-Drive against hotspot motifs in primates implicates the *PRDM9* gene in meiotic recombination- Science 327: (#5967, 2/12) 876-879

Parvanov E.D., P.M. Petkov, K. Paigen- 2010-Prdm9 controls activation of mammalian recombination hotspots- Science 327: (#5967, 2/12) 835

Crossing over occurs alot during meiosis, but it also occurs during **mitosis**. In some cases this has been associated with cancer.

Hartwell L., L. Hood, M.L. Goldberg, A.E. Reynolds, L.M. Silver, R.C. Veres- 2000-Mitotic recombination and cancer formation. Pgs. 114-115, in Chapter 4 of <u>Genetics: From Genes to Genomes</u>- McGraw Hill. Boston, MA.

Raposo A., C.R. Caralho, W.C. Otoni- 2004-Statistical and image anlaysis of sister chromatid exchange in maize- Hereditas 141: 318-322

And some animals have systems that limit the amount of crossing over that is done. In this case *C. elegans* has a system that typically allows just one crossover event between each homologous pair of chromosomes per meiosis. The system is called **crossover interference**.

> Youds J.L., D.G. Mets, M.J. McIlwraith, J.S. Martin, J.D. Ward, N.J. O'Neill, A.M. Rose, S.C. West, B.J. Meyer, S.J. Boulton- 2010-RTEL-1 enforces meiotic crossover interference and homeostasis- Science 327: (#5970, 3/5) 1254-1258

Plants and fungi make spores by meiosis, this pattern is called sporic meiosis. In one of the following articles moss (a bryophyte) is noted for holding the spores made by meiosis in an **ordered-linear tetrad** arrangement, which is useful for genetic studies.

> Rim Y.W., P.R. Beuselinck- 1996-Cytology of 2N pollen formation and pollen morphology in diploid *Lotus tenuis* (Fabaceae)- American Journal of Botany 83: (#8) 1057-1062
>
> Shimamura M., H. Deguchi, Y. Mineyuki- 1998-Meiotic cytokinetic apparatus in the formation of the linear spore tetrads of *Conocephalum japonicum* (Bryophyta)- Planta 206: 604-610

This article describes aspects of **segregation** during meiosis I.

> Brar G.A., A. Amon- 2008-Emerging roles for centromeres in meiosis I chromosome segregation- Nature Reviews Genetics 9: (#12, Dec.) 899-910

This article notes that failure of proper **segregation** during meiosis can result in non-disjunction.

> Wu C., V. Singaram, K.S. McKim- 2008-*mei-38* is required for chromosome segregation during meiosis in *Drosophila* females- Genetics 180: (#1, Sept.) 61-72

Here are articles with more information on Mendel and **Mendelian genetics**. Some of them deal with identifying the actual genes that code for the **pea traits** that Mendel studied.

> Armstead I., I. Donnison, S. Aubry, J. Harper, S. Hörtensteiner, C. James, J. Man, M. Moffet, H. Ougham, L. Roberts, A. Thomas, N. Weeden, H. Thomas. I. King- 2007-Cross-species identification of Mendel's I locus- Science 315: (1/5) 73
>
> Fairbanks D.J., B. Rytting- 2001-Mendelian controversites: A botanical and historical review- American Journal of Botany 88: (#5) 737-752
>
> Hartwell L., L. Hood, M.L. Goldberg, A.E. Reynolds, L.M. Silver, R.C. Veres- 2000-Mendel's breakthrough: Patterns, particles, and principles of heredity- Chapter 1, pgs. 10-37, in, <u>Genetics: From Genes to Genomes</u>- McGraw Hill. Boston, MA.
>
> Wang T.L., T.Y. Bogracheva, C.L. Hedley- 1998-Starch: As simple as A, B, C?- Journal of Experimental Botany 49: (#320) 481-502

Recall that one feature of animals is that they have **arrested meiosis**. Here is an article that examines aspects of meiotic arrest.

Homer H., L. Gui, J. Carroll- 2009-A spindle assembly checkpoint protein function in prophase I arrest and prometaphase progression- Science 326: (#5955, 11/13) 991-994

BIO 108   2010
Day 2, Lecture 4, Title: Post-Mendelian Genetics.
Text Readings: Campbell et al. (2008), pgs. 271-281.

Topics to cover:

  Classical Mendelian Model
    Pedigree Analysis
    Review Gene Expression
  Post-Mendelian Models
    Incomplete Dominance
    Codominance
    Multiple Alleles
    Pleiotropy
    Epistasis
    Polygenic Traits
    Environmental Influences
    Cytosolic Inheritance
  Summary

Classical Mendelian Model
  Mendel picked his research species and traits carefully,
    so narrowed things down to something he could make sense of...
  This model has been modified, review some of its features for comparison
Mendelian Genetics assumes (amongst other things...):
    genes, one per characteristic and no more than two allelic types in a population
    genes do not interact, in how they assort or are expressed
      independent assortment occurs between each pair of genetic factors
    dominant/recessive relationships only between alleles
    and each genotype faithfully shows up as its phenotype
Examples that fit in Mendelian genetics are numerous, here are a few...
  Recessive inherited illness, note can be hidden in heterozygote state = "carriers"
    cystic fibrosis
      4% of human population are carriers of the allele for this
      chloride ion channel error, produces higher [Cl^-] extracellular
      water follows by osmosis
      fluid/mucus filled lungs, pancreas, etc, which promotes disease
    Tay Sachs disease
      error in lipid metabolism, → nerve damage
  Dominant inherited illness
    Huntington's disease, results in degraded nervous system

Pedigree Analysis

Can use this to determine features of a genetic trait

Example of Pedigree analysis of humans

        example widow's peak and attached earlobe

                Fig. 14.15, pedigree analysis

        Note designation of males and females, and those with phenotype of a trait

Do two examples.

Overhead, pedigree.tif

        Do a recessive trait, like cystic fibrosis

        Do a dominant trait, like Huntington's

        (see question #16, pg. 285, as a practice example

                Fig. 14_UNO5GP-L -pedigree, alkaponuria, pedigree analysis)

Review Gene Expression

How does genetics relate to phenotype?

What do genes code for? (draw following out)

        Fig. 17.3, transcription and translation

        Flow of information from DNA to RNA to proteins

                proteins do things, make things and produce phenotype...

        Proteins occur in pathways of biosynthesis, like glycolysis, Krebs cycle, etc...

        If diploid could have two alleles for each gene

                so up to two versions of the protein, may work differently

                error in gene, makes non-functional protein

        Dominant phenotype, one copy of good gene is enough

        Recessive phenotype, need two bad copies of gene to block

Post-Mendelian Models

        Mendel kept things simple, so he could find a pattern

        Real world science begins with a simple model and adds to/modifies it to improve it

        Following are some of the modifications built onto the Mendelian model

Incomplete Dominance

        Mendel assumed strict Dominant/recessive relations between alleles for a gene.

        There are exceptions to this...

        Example of Incomplete dominance

                Fig. 14.10, snapdragon incomplete dominance

                Note phenotype of heterozygote is intermediate between two homozygous states

                Work through amount of enzyme made by each genotype

                        if product formation is limited by concentration of enzyme

                        then can get the intermediate phenotype

                Note that this is a sort of blending inheritance?

                      So pre-Mendelian idea was partly right?

Codominance
    another case of not clear dominant/recessive state
    heterozygous state (I^aI^b) is NOT intermediate between two homozygous states
    Example: human blood types, note relationships between the alleles
        Fig. 14.11, human blood types
        I^a and i   are dominant/recessive
        I^b and i   "   "
        I^a and I^b are codominant
    AB blood type is not intermediate, an entirely new phenotype
    Note anti-A and anti-B antibody reactions against blood of various types
        Fig. 14_11xABObloodtypes_XLP, blood type test
        so the phenotypes do differ...

Multiple Alleles
Mendel worked with just two allelic versions of each gene
Alleles are in populations, instead of just individuals
    multiple alleles (i.e. more than two) can occur in a population
    though only two alleles in an individual
See this in blood type example, where have three types of alleles
    only two can be in any one person, but all three can occur in the population
Many genes have dozens of allelic versions in the population...

Pleiotropy
Mendel assumed one genotype influenced one phenotype
In Pleiotropy see multiple phenotypic effects from one genotype
    Ex: Sickle-cell disease
        Fig. 14.15, (Campbell and Reece, 2002) sickle-cell disease phenotypes
    Another example:
        mutation in gene for tubulin, alters microtubules, alters cilia, alters flagella,
            alters spindle apparatus
            consider all the phenotypic effects this could have...

Epistasis
Mendel did not consider any interaction between genes in affect on phenotype
Epistasis, one genotype influences expresson of another genotype...
Draw out biochemical pathway
    upstream genes, vs. downstream genes in a pathway
    gene upstream has an effect on all genes downstream
Example: mouse color
    Fig. 14.12, epistasis
    note change in ratio gotten from classic dihybrid 9:3:3:1

Polygenic Traits
Mendel assumed that one genetic factor coded for one trait
Polygenic inheritance, see multiple genes all influencing one phenotype
        many gene products contribute to one phenotype
                remember that each gene can have multiple allelic versions as well...
                Fig. 14.13, polygenic inheritance
        skin color
                hypothesis: multiple pigment pathways?
                so many genes contribute through multiple to one common phenotype

Environmental Influences
Mendel assumed that the genotype is always faithfully displayed in the phenotype
but Enviromental influences may alter gene expression
        Fig. 14.14, environmental influences
        hydrangea flower color altered by soil pH
        One thing to have genetic potential
                another thing to achieve that potential
        Environment influences gene expression, and/or protein activity.
        Growth and development factors matter
        Even after mature can change phenotype

Cytosolic Inheritance
Mendel assumed up to two copies of each gene in a diploid cell
This is often true for nuclear genes, but not all genes are in the nucleus
Fig. 6.9, animal and plant cells
DNA is also found in mitochondria and in chloroplasts
        and genetic factors may also be in other organelles...
These organelles can exist in hundreds to thousands per cell
So can be more than two versions of a gene per cell
Genes in the cytosol must segregate during cell division,
        but assortment can be very different from Mendelian...
In some cases see cytosolic genes inherited from maternal line, not from paternal line

Summary
So Mendelian genetics is a simple starting point
        he had to do this, otherwise it would have been too complicated to model,
        but many modifications made to that initial model
This is typical of science.
Other genetic aspects exist... More modifications to the model will be made...

Objectives:

Mendel did not explain everything about genetics. Know the conditions that must be met for Mendelian genetics to apply so that you can describe where Mendel's model of genetics ends and where the model has been expanded beyond his to explain additional observations.

Are all phenotypic traits under strict genetic control? What is an example of an environmental influence on phenotype?

Be able to work with pedigree data to determine if a trait is dominant or recessive and the probable genotypes of ancestors.

How do the following differ: Strict dominant/recessive, incomplete dominance, and codominance? Be able to describe the genetic basis and recognize examples of: Multiple alleles, pleiotropy, epistasis, and polygenic traits. Do these always result in phenotypic ratios different from what would be expected from the Mendelian model? What might be happening at the molecular level that could account for each of these situations? Which of these did Mendel describe and which are post-Mendelian?

What is cytosolic inheritance, in what way does it differ from Mendelian genetics, and in what way is it consistent with Mendelian genetics?

For review see self-quiz questions #5-8, and 11-19 on pgs. 284-285 of your text. (See pg. 283 for some suggestions on how to approach genetic problems, and remember the answers to all these questions are given in the back of the text.)

Needed overheads and items:

Fig. 14.15, pedigree analysis
pedigree.tif, for dominant / recessive analysis
Fig. 14.UNO5GP-L -Pedigree, Alkaptonuria, for pedigree analysis
Fig. 17.3, transcription and translation
Fig. 14.10, snapdragon incomplete dominance
Fig. 14.11, human blood types
Fig. 14_11xABObloodtypes_XLP
Fig. 14.15, (Campbell and Reece, 2002) sickle-cell disease phenotypes
Fig. 14.12, epistasis
Fig. 14.13, polygenic inheritance
Fig. 14.14, environmental influences
Fig. 6.9, animal and plant cells

Handout:

Lecture 4 - Handout- Pedigree Analysis.stm

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008n-Mendel and the gene idea. Chapter 14. Pages 271-281. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-Biology, 6th edition, Figure 14.13. Benjamin Cummings Press. San Francisco, CA.

Related Issues:

Here are some studies that made use of **pedigree** analysis, amongst other techniques...

Mani A., J. Radhakrishnan, H. Wang, A. Mani, M-A. Mani, C. Nelsen-Williams, K.S. Carew, S. Mane, H. Najmabadi, D. Wu, R.P. Lifton- 2007-LRP6 mutation in a family with early coronary disease and metabolic risk factors- Science 315: (3/2) 1278-1282

Nurnberger J.I. jr., L.J. Bierut- 2007-Seeking the connections: Alcoholism and our genes- Scientific American 296: (#4, April) 46-53

For aspects of **pleiotropic** effects:

Flatt T., D.E.L. Promislow- 2007-Still pondering an age-old question- Science 318: (#5854, 11/23) 1255-1256

For a consideration of the possibilities and potential problems with **genetic testing** see:

Couzin J- 2008-Once shunned, test for Alzheimer's risk headed to market- Science 319: (#5866, 2/22) 1022-1023

Couzin J- 2007-Amid debate, gene-based cancer test approved- Science 315: (2/15) 924

Couzin J., J. Kaiser - 2007-Closing the net on common disease genes- Science 316: (5/11) 820-822

Frayling T.M., N.J. Timpson, M.N. Weedon, E. Zeggini, R.M. Freathy, C.M. Lindgren, J.R.B. Perry, K.S. Elliott, H. Lango, N.W. Rayner, B. Shields, L.W. Harries, J.C. Barrett, S. Ellard, C.J. Groves, B. Knight, A-M. Patch, A.R. Ness, S. Ebrahim, D.A. Lawler, S.M. Ring, Y. Ben-Shiomo, M-R. Jarvelin, U. Sovio, A.J. Bennett, D. Melzer, L. Ferrucci, R.J.F. Loos, I. Barroso, N.J. Wareham, F. Karpe, K.R. Owen, L.R. Cardon, M. Walker, G.A. Hitman, C.N.A. Palmer, A.S.F. Doney, A.D. Morris, G.D. Smith, The welcome Trust Case Control Consortium, A.T. Hattersley, M.I. McCarthy- 2007-A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity- Science 316: (5/11) 889-894

Epistasis implies that one gene's expression depends on the state of another gene, here is a study that attempts to map the **epistatic** relations between genes in yeast. And another study of epistasis seen in carotenoid synthesis in carrots.

Just B.J., C.A.F. Santos, B.S. Yandell, P.W. Simon- 2009-Major QTL for carrot color are positionally associated with carotenoid biosynthetic genes and interact epistatically in a domesticated X wild carrot cross- Theoretical and Applied Genetics 119: (#7, Nov) 1151-1169

Roguev A., S. Bandyopadhyay, M. Zofall, K. Zhang, T. Fischer, S.R. Collins, H. Qu, M. Shales, H-O. Park, J. Hayles, K-L. Hoe, D-U. Kim, T. Indeker, S.I. Grewal, J.S. Weissman, W.J. Krogan- 2008-Conservation and rewiring of functional modules revealed by an epistasis map in fission yeast- Science 322: (35900, 10/17) 405-410

Here is a study of hair color in dogs that find that there are **multiple genes** all influencing this one trait. (They do not say if while studying the hair of the dog it bit them!) The other study looks at the influence of multiple genes on the final phenotype seen in yeast.

Cadieu E., M.W. Neff, P. Quignon, K. Walsh, K. Chase, H.G. Parker, B.M. VonHoldt, A. Rhue, A. Boyko, A. Byers, A. Wong, D.S. Mosher, A.G. Elkahoun, T.C. Spady, C. André, K.G. Lark, M. Cargill, C.D. Bustamante, R.K. Wayne, E.A. Ostrander- 2009-Coat variation in the domestic dog is governed by variants in three genes- Science 326: (#5949, 10/2) 150-153

Dowell R.D., O. Ryan, A. Jansen, D. Cheung, S. Agarwala, T. Danford, D.A. Bernstein, P.A. Rolfe, L.E. Heisler, B. Chin, C. Nislow, G. Giaever, P.C. Phillips, G.R. Fink, D.K. Gifford, C. Boone- 2010-Genotype to phenotype: A complex problem- Science 328: (#5977, 4/23) 469

One exception to Mendelian genetics are the genes found in the mitochondria and chloroplast. They undergo non-Mendelian **cytosolic inheritance**.

Hartwell L., L. Hood, M.L. Goldberg, A.E. Reynolds, L.M. Silver, R.C. Veres- 2000- The chromosomes of organelles outside the nucleus exhibit non-Mendelian patterns of inheritance- Chapter 14, pgs. 501-526, in, <u>Genetics: From Genes to Genomes</u>- McGraw Hill. Boston, MA.

Mayfield S.P., C.B. Yohn, A. Cohen, A. Danon- 1995-Regulation of chloroplast gene expression- Annual Review of Plant Physiology and Plant Molecular Biology 46: 147-166

Reith M- 1995-Molecular biology of Rhodophyte and Chromophyte plastids- Annual Review of Plant Physiology and Plant Molecular Biology   46: 549-575

Shoubridge E.A., T. Wai- 2008-Side stepping mutational meltdown- Science 319: (#5865, 2/15) 914-915

Timme R.E., J.V. Kuehl, J.L. Boore, R.K. Jansen- 2007-A comparative analysis of the *Lactuca* and *Helianthus* (Asteraceae) plastid genomes: Identication of divergent regions and categorization of shared repeats- American Journal of Botany 94: (#3) 302-312

Wallace D.C- 1997-Mitochondrial DNA in aging and disease- Scientific American 277: (#2) 40-47

For a review of other aspects of **non-Mendelian genetics** see:

Hartwell L., L. Hood, M.L. Goldberg, A.E. Reynolds, L.M. Silver, R.C. Veres- 2000- Extensions to Mendel: Complexities in relating genotype to phenotype- Chapter 2, pgs. 38-69, in, <u>Genetics: From Genes to Genomes</u>- McGraw Hill. Boston, MA.

BIO 108   2010
Day 2, Lecture 5, Title: Chromosome Structure.
Text Readings: Campbell et al. (2008), Chap. 15.

Topics to cover:

> **Chromosomal Theory**
> > **Sex Linkage**
> **Determining Linkage between two genes**
> > **Linked vs. Unlinked Models**
> > **Crossing over and Map units**
> > **Physical vs. Genetic Linkage**
> **Uses of Genetic Mapping**
> **How Chromosome Structure can change**
> **Other Oddities**

Chromosomal Theory
Now will consider arrangements of genes in chromosomal structure.
This is building further on Mendel's model, but it also raises many features not anticipated by it,
        and so we continue outside of his model.
Will look at how to describe gene linkage and some approaches to the study of it, and then get
        into a few other chromosomal features.
By early 1900s researchers could relate:
        Principle of segregation to behavior of chromosomes during mitosis and meiosis.
        Principle of independent assortment to behavior of homologous pairs of chromosomes
                and of recombinant double-chromatid chromosomes during meiosis.
So thinking was that chromosomes contain genetic material
        Proposed in 1902, by Sutton and others, Chromosomal theory of inheritance
Needed a system to test this model, at that time just a correlation.

> **Sex Linkage**
> Could study inheritance of traits in fruit flies
> *Drosophilia melanogaster* as an experimental system.
> Drosophilia2.jpg, fig. 21.4, Hartwell etal. 2000, life cycle
> Why use them? note life cycle...
> > easy to grow and control mating, fast generation time,
> > several distinct ecotypes, with good genetic diversity readily available
> > genome has just 4 types of chromosomes...
> Were also able to see chromosomes, karyotype, by squashing and staining
> > Fig. 15x4, (Campbell and Reece, 2005) Klinefelter Karyotype,
> >   human chromosomes
> > already knew of X and Y chromosomes and sex determination
> > Fig. 15.5, SEM of X and Y chromosomes

1

Morgan's experiments with fruit flies

        Fig. 15.3, red and white eyed flies

        Found a white eyed mutant, so studied inheritance of its traits

        Did cross, note initial model implied each sex had two copies of this gene

            noticed this trait was associated with sex chromosome

        So altered model to put gene on the sex chromosomes

            initially had a version on both the X and Y chromosomes

        Discovered that no version of the gene found on Y chromosome

            yet another modification of the Mendelian model...

            so male fruit flies are effectively haploid

        Fig. 15.4, Wild type X Mutant cross, sex linkage

        Thus could relate a genetic factor to the XY pair of chromosomes

            so a specific genetic factor is in this homologous pair of chromosomes

            this was first instance of data directly supporting this association

            traits linked to Sex chromosomes, sex linkage

        Supports chromosomal theory: genes in the chromosomes

Determining Linkage between two genes

        So if can determine which genes are in one homologous pair (XY)

            then can we determine if two genes are together in a homologous pair?

        Thousands of genes in fruit flies, four types of chromosomes (1N =4)

            small number of types of chromosomes a good thing...

            this made fruit flies a good model organism for these studies

Linked vs. Unlinked Models

Example cross two pure breeding lines, then do test cross of dihybrid

        Fig. 15.9, dihybrid testcross, upper part of figure

        So do a cross: $b^+b^+vg^+vg^+$ X bbvgvg   of the two true breeding lines

            get the dihybrid and do a test cross of it

        F1 testcross:

            Gray with normal wings vs. black with vestigal wings

            b^+b vg^+vg X   bb vgvg

        Show linked model (draw chromosomes, note convention use of "/")

            b^+ vg^+/b vg X b vg/b vg

            predicts just two offspring types, expect to get a 1:1 ratio

        Show unlinked model (draw chromosomes)

            b^+b vg^+vg X bb vgvg

            predicts four type of offspring, expect   1:1:1:1

        When do the actual cross: Get neither of the above expected results!

        Fig. 15.9, dihybrid testcross, lower part of figure

        Critical issue: When our models fail, then we have a chance to learn!

Crossing over and Map units
So to account for results, need a new phenomenon.... typical of science
In this case, the new phenomenon is crossing over
Crossing over, this typically occurs between genes on the same chromosome...
    Occurs at Chiasmata structures, genetic exchange during Prophase I of meiosis
    It is extremely common during meiosis, but also occurs rarely in mitosis,
        about $1/10^6$.

    In this cross, crossing over will alter expected gametes from just one parent
    Fig. 15.10, Linkage in Drosophilia, chromosomes, upper part
        Note the cross over event in the female
            (male fruit flies do not have crossing over during meiosis)
        This produces new recombinant types, and non-recombinant types
    Note the original parental combinations, and new combinations of phenotypes
        Notice that one cross over event alters only two out of four chromatids
    Fig. 15.10, Linkage in Drosophilia, chromosomes, lower part
    Map unit: defined in terms of percentage of recombinants products relative to the
        total number of produces of meiosis.
        (Not defined as the percentage of cross over events!)
    Note that this is a measure of recombination products,
        which is proportional to the likelyhood of crossing over (i.e. 2X map unit =
        percent of crossing over during meiosis)
        So a map unit is a probability, it is not an actual distance
            but the more DNA between two genes the more map units between
    For this case:
    $[(206 + 185)/(2300)]100$ = % recombinants = map unit (mu) = centimorgans
                    17.0 = map units
    Maximum value of map unit we can detect is 50 map units.... see why?
        If every meiosis had a cross over event between these genes.
        Then would expect the following ratio:
            1 b^+vg^+ : 1 bvg : 1 b^+vg : 1 bvg^+
        This would give:
            1650 recombinants, and 1650 non-recombinants
            this gives a map unit of 50
        Notice that this is the same ratio expected from the model
            of independent assortment!
        So with enough crossing over can get independent assortment
            genes on different chromosomes are very unlinked...

Physical vs. Genetic Linkage
If have crossing over every time, then get 1:1:1:1 ratio of gametes
   this looks like unlinked model.
So high rates of crossing over look unlinked, this occurs when >50 map units
This implied that there are two types of linkage.
   Physical linkage:
     If two genes are in same DNA molecule, then in same chromosome and
     Physically Linked
   Genetic linkage:
     Even if physically linked, the two genes could be >50 map units apart, and
      so independently assort
     if <50 map units apart, will not independently assort
     in this case they are also Genetically Linked
     Only genes within 50 map units of each other can be genetically linked
linkage.tif
   Notice that three possible arrangements for two genes (g and t) shown
So genetic linkage of two genes assumed that they are physically linked
   but two genes can be physically linked and still be genetically unlinked.
   Thus independent assortment can occur between physically linked genes.

Uses of Genetic Mapping
  So can determine linkage of pairs of genes in chromosomes, and map units between them
  This can allow for determining several things
    Fig. 15.11, linkage
    Which genes are present in which homologous pair of chromosomes
    And can determine the order of genes along that chromosome
  This allows production of genetic maps, representing order of genes in a chromosome
    Fig. 15.12, Drosophilia genetic linkage map
    Note that this one chromosome has 104.5 map units along its length!
    Any two genes over 50 map units apart will independently assort
    so even genes that are physically linked can independently assort
    Some human genes have over 400 mu along their length
    This increases the amount of genetic varation in the next generation
      increases the genetic recombinations made during meiosis...
    By putting genes into distinct homologous pairs limit the recombining sets
  When do enough of these studies, can locate many genes on all of the chromosomes
    Drosophilia3.jpg, fig. 4.15, Hartwell etal. 2000, gene map
    note the number of map units along these chromosomes...
    obviously this only works for genes that meet certain conditions
      need distinct alleles that produce phenotypes we can discern... etc...

Genetic Maps
        With such a genetic map can predict likelyhood of crossover events
        monitor gene order for changes in chromosomal structure,
                i.e. evolutionary changes?
        Does the arrangement of genes on a chromosome influence their expression?
Physical Maps
        another type of chromosome map, based not on recombination products
        based on number of nucleotides along length, possible with DNA sequencing
        The challenge here is to locate each gene in this DNA sequence
                different regions of DNA cross over alot, other regions very little
                the area around the centromere tend not to cross over much...
Now often have both physical and genetic maps of many organism's genomes
        this gives us two different ways to look at the same chromosomes

How Chromosome Structure can change
        Changes can occur due to viruses, transposition, and other processes...
        Unequal crossing over, can cause a deletion or gene duplication in chromosome.
                Obviously this often has lethal effects, or causes very unusual phenotypes.
        But can be detected by changes in map unit distances...
        Fig. 15.15, chromosome alterations
        can occur via mutations of various types
                deletion, or insertion
                duplication, can produce new copies of genes, available for new uses?
                        Individual humans differ in number of copies of certain genes
                                may have several copies of a gene in one part of a chromosome
                                someone else may have just one copy of that gene there...
                      so this sort of variation can exist within a species
                inversion, alters gene arrangement and perhaps expression?
                translocations, can occur between different homologous pairs
                      Fig. 15.17, translocation
                      in some cases a chromosome translocation can induce cancer
                      can detect this with specific probes of karyotype
                      Fig. 15.x3, (Campbell and Reece, 2005) translocation staining
        So if chromosome structure can change, this should be involved in species formation?
                So if look at chromosome structures of different species,
                      should be able to determine how they are related?
                This is yet another means to reconstruct evolutionary relationships...

Other Oddities
Nondisjunction, during meiosis or mitosis
        Fig. 15.13, nondisjunction
                an error in segregation so that full sets are not separated
        Can produce trisomy, three copies of a chromosome
                Can produce Down's Syndrome
                Fig. 15.16, Down's Syndrome
                Can confirm this by looking at karyotype from an individual's cells
X chromosome inactivation
        See this in many mammalian species. One of the two X chromosomes condenses
                Stays in condensed state, even in interphase, so genetically silent
                This structure is called a "Barr body" after the discoverer
        Barr bodies form in each female human cell.
        Produces Calico Cats
        Fig. 15.x2, (Campbell and Reece, 2005) Calico Cat
        Fig. 15.8, X chromosome inactivation
        This makes each cell haploid in expression of genes on X chromosomes,
                but can share products with other cells....
                so mammalian females are a chimera, with groups of distinct phenotypes
                males have just one X chromosome, so can not do this...
        Note, this is yet another exception of Mendelian genetics...
Genomic imprinting, can alter gene expression
        Fig. 15.18, genomic imprinting
        methylation of certain regions of the DNA occurs
        influences gene expression, some of this is altered before entry into meiosis or mitosis
        Here a heterozygous genotype is present, expect to see just dominant phenotype
                but due to methylation, could silence the dominant allele,
                    and so get recessive phenotype
                or could silence recessive allele, and see dominant phenotype
        sperm and egg can deliver different methylation patterns from parents
                so we do not just inherit genes, also inherit certain gene modifications....
        yet another modification of Mendelian genetics....

So we can associate genes with chromosomes.
        And can determine the order of the genes on the chromosomes.
        The chromosomal theory is supported. But, not this does not tell us everything.

["Logically, a good way to tell how the work is going is to listen in the corridors. If you hear the word, "Impossible!" spoken as an expletive, followed by laughter, you will know that someone's orderly research plan is coming along nicely."
        -Lewis Thomas-]

Objectives:

What is the chromosomal theory and upon what evidence is it based? Be able to describe sex linkage. Given pedigree data or the results of a cross be able to determine if a trait is likely to be sex linked or not.

Be able to compare the phenotype ratios for the next generation based on the following cross according to the linked versus unlinked genetic model. (Assume each gene codes for a distinct phenotype each in a strict dominant/recessive pattern of its alleles.) Draw how the chromosomes would appear at metaphase I of meiosis for the linked versus the unlinked model.

           Cross with genes Unlinked: BbAa X bbaa
           Cross with genes Linked: BA/ba X ba/ba

If two genes exist in the same chromosome but are separated by 75 map units will these two genes appear to be genetically linked or not? Will they assort independently during meiosis or not? What is the difference between genetic linkage and physical linkage? Understand the definition of the map unit and how it is not an actual distance. Be able to calculate a map unit from a set of data, or be able to predict what would result in a cross if given a map unit between two genes.   Be able to use map units to arrange genes in their proper order on a chromosome.

Why does the percentage of meioses with crossover events in them differ from the map unit? If you are given the percentage of crossover events can you calculate the expected number of recombinant products of meiosis? Be able to describe a crossover event in terms of which chromatids are actually being altered and which are not altered, and relate this to the production of recombinant or non-recombinant offspring.

Be able to describe several ways that the structure of a chromosome can be altered.

Describe X chromosomal inactivation, non-disjunction, and genomic imprinting: Be able to give examples of how each could alter the phenotype of an organism. Consider whether these are consistent with the model of genetics as proposed by Mendel.

For review see self-quiz questions #1-6, and 9-14 of chapter 15.

Needed Figures:

Drosophilia2.jpg, fig. 21.4, Hartwell etal. 2000, life cycle
Fig. 15x4, (Campbell and Reece, 2005) Klinefelter Karyotype, human chromosomes
Fig. 15.5, SEM of X and Y chromosomes
Fig. 15.3, red and white eyed flies
Fig. 15.4, Wild type X Mutant cross, sex linkage
Fig. 15.9, dihybrid testcross, upper part of figure
Fig. 15.9, dihybrid testcross, lower part of figure
Fig. 15.10, Linkage in Drosophilia, chromosomes, upper part
Fig. 15.10, Linkage in Drosophilia, chromosomes, lower part
Linkage.tif
Fig. 15.11, linkage
Fig. 15.12, Drosophilia genetic linkage map
Drosophilia3.jpg, fig. 4.15, Hartwell etal. 2000, gene map
Fig. 15.15, chromosome alterations
Fig. 15.17, translocation
Fig. 15x3, (Campbell and Reece, 2005) translocation staining
Fig. 15.13, nondisjunction
Fig. 15.16, Down's Syndrome
Fig. 15x2, (Campbell and Reece, 2005) Calico Cat
Fig. 15.8, X chromosome inactivation
Fig. 15.18, genomic imprinting

Handout:
Handout - Lecture 5- Linkage.stm
(Linkage.tif)

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For more about **X chromosome inactivation** in mammals and the resulting **Barr bodies** see:

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For how **genomic imprinting** and how changes in **histone methylation** or **acetylation** can operate to control gene expression and influence development or aging see:

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BIO 108   2010
Day 2, Lecture 6, Title: Fungal Diversity.
Text Readings: Campbell et al. (2008), Chap. 31.
Topics to cover:

>   **Common Features of Fungi**
>   **Life Strategy and Ecological Role**
>   **Life Cycle**
>   **Three Major Phyla**
>>   **Zygomycota**
>>   **Basidiomycota**
>>   **Ascomycota**
>>>   **Ordered Tetrads**
>   **Our Uses of Fungi**
>   **Summary**

Common Features of Fungi
>   We continue our coverage of the diversity of life:
Kingdom Fungi:
>   Fig. 31.2   Basidiomycete, and mycelia
>   Some common features:
>>   note hyphae that come together to make up mycelia
>>   heterotrophic eukaryotes, do extra-organismal digestion, secretes enzymes
>>   chitinaceous cell walls (just like some animals who have chitin...)
>>   distinct life cycles, no multicellular diploid phase...
>>>   typically have a distinct dikaryotic (N+N) phase in the life cycle
>>   disperse spores from place to place
Life Strategy and Ecological Role
Life strategy and ecological role
>   many physiological processes covered by their high surface area to volume ratio
>>   Fig. 31.3,   Hyphae
>>   gas exchange, across high SA, so no specialized structures
>>>   in some cases can live via fermentation, so can survive lack of O_2
>>   waste removal, also across high SA
>>   nutrient procurement
>>>   Secrete digestive enzymes, then absorb monomers
>>>>   saprophytic fungi, digest dead organic matter, decomposers
>>>>   parasitic fungi, take on living cells of a living organism
>>>   Fig. 31.4,   predation, parasites
>>>   predatory, catch animals (nematodes) and then digest them...
>>>   symbiosis, can supply food to some species, mycorrhizae...
>>>   Fig. 31.14, (Campbell and Reece, 2002)   mycorrhizae
>>>>   can also have symbiosis with some algae

Ecological role
    In addition to being major decomposers, they also help establish life in new places
    Pioneers can live on bare rock, lichen, an association of fungi and algae or cyanobacteria
    Fig. 31.23   lichen
    Fig. 31.24   lichen, colorized SEM
        a symbiotic relationship, important for colonizing on bare rock...
        between fungus and photosynthetic bacteria or eukaryotic alga
        another example of a symbiosis
        See these on cemetary stones, on tree bark
            are good indicators of air quality, pollution tends to kill lichen

Life Cycle
    Review general fungal life cycle
    Fig. 31.5,   fungal life cycle
        Note that this figure does NOT show mitotic divisions, where are they?
        Reproduction
            asexual and/or sexual patterns done by various species
            some have asexual option, used if conditions are good.
        note, all have N+N (dikaryotic) stage of some type
        use of spores for dispersion
        most of mycelia live underground, or in another organism, so protected...
        Sexual life cycle as has meiosis and fertilization.
            produces spores sexually, and makes zygotes sexually
        revisit the disruption of fertilization into very distinct stages
            plasmogamy and karyogamy, with mitotic cell divisions inserted

Three Major Phyla
    Fig. 31.11   Phylogeny of fungi
    Five major groups, won't get into Chytridiomycota or Glomeromycota
    the three other phyla are characterized by structures where meiosis is done

    Zygomycota
    Fig. 31.13   Zygomycota life cycle, bread mold
        coenocytic, so all one cell
        has zygosporangium, in which many nuclei share a cytosol after plasmogamy
            tough cell wall, can go dormant like this...
        Fig. 31.13, zygosporangium
            nuclei do karyogamy, and then have diploid nuclei
            these do meiosis and then cytokinesis to become zygospores

Fig. 31.14, *Pilobolus* aiming sporangia
    One species live in dung of cows, how to get back into cow's gut?
    cows eat grass, so fungus has area behind sporangia swell by osmosis and burst
    this propells fungal spores to surrounding grass, taken up by cow
        Note: nematodes that are parasites in cows
                also need to get back into cows
                sense chemical features of sporangia, ride on it
                so gets back into cow as well...

Basidiomycota
Fig. 31.20, fairy ring
    Each "Fairy ring" represents one individual organism,
        one spore grows out hyphae underground
    gets to a "mature" stage, where can form dikaryotic hyphae
        this grows out from one spot, and puts up basidiocarps at the edges
    so get a ring of mushrooms...
Fig. 31.18, basidiomycetes
Fig. 31.19   Basidiomycota life cycle
    defined by basidia, structure in which meiosis is done and spores made
    "mushroom" is above ground "fruiting" structure to release spores, a basidiocarp
    most of body of organism is below ground
    note, a mushroom is N+N, think about that when you eat one...
Fig. 28.15 (Purves etal., 1998) basidiomycota3.jpg, basidia
    point out basidium and basidiospores attached to it.

Ascomycota
Fig. 31.16, ascomycetes
Fig. 31.17, (Campbell and Reece, 2005) Ascomycota life cycle
    has asci (singular: ascus) in which meiosis is done and spores are made
    Notice again the N+N stage that forms a "fruiting body", the ascocarp
    the hyphae live underground

    Ordered Tetrads
    Fig. 31.10, (Campbell and Reece, 2005) Asci in perithecium
    benefits of order ascus to genetic analysis
        spores are haploid, what is advantage of this?
        order ascus with meiotic products arranged as four chromatids in meiosis I
        show how the spore arrangmenets relate to meiosis
    Ordered tetrad gives more information about events during meiosis than normal.

Consider two asci
    Gray Black Cross no crossing over.jpg (Glase 2006; fig. 11.5)
    Gray Black Cross with crossing over. jpg (Glase 2006; fig. 11.4)
    created from a cross of black and gray strains of *Sordaria fimicola*
    zygotes in both cases are g^+g
    Get 8 spores in each case
    In each case 4 spores are gray, and 4 are black
    If popped the asci and just looked at spores the produces are similar
    But if hold spores in asci, then can tell if crossing over occurred.
        this is additional information that you would not get if used
        fruit flies, mice, or anything that has no ordered tetrad...

Our Uses of Fungi
Antibiotics
    Fig. 31.26, Penicillium
    Penicillium sp. an "imperfect" fungus (also called deuteromycetes)
        meaning no sexual stage as been found yet
    Fungi secrete toxins to kill other soil inhabitants
        They then have sole use of items they digest
        or can digest the organisms they kill
    We use some of these as antibacterial compounds for our benefits.
    So fungi are a major source of antibiotics we use in our medicines
Genetic studies
    Already described uses of fungi in studies of linkage
        Advantages of haploid stages, very rapidly growing, easy to reproduce
        Crossover studies and mapping genes
    Current use of YACs: Yeast artificial chromosomes
        Can put in up to 1 Mbp of genetic material
        Then put it in yeast, and have it expressed in eukaryotic system
        Very useful for studying of eukaryotic genes in entire pathways at once
        Many human genes and genetic elements have been put into yeast for study
Agricultural issues:
    often fungi are pathogens of crops and of trees..., a downside
        Fig. 31.25, rusts on plants
        though, corn smut is also considered a delicacy...
    mycorrhizal associations with plants
        Fig. 31.18, (Campbell and Reece, 2002) mycorrhizae
        many plants would not grow well without associations with fungi
Fermentative uses
    Fig. 31.7, yeast
    Yeast, *Saccharomycetes cerevisiae*
    Baker's, and Brewer's yeast are two distinct strains
    We force them to do fermentation and make ethanol, by depriving them of O_2

Summary

See table on pg 652 (Campbell et al., 2008) features of fungal groups

      review where spores are made in each group, spore forming structures define them

      N+N stages are the major distinctive stage of fungi

            but also have 1N hypha as well before get to the dikaryotic stages

      role as decomposers, without them most ecosystems would have essential minerals

            locked up in organic matter, and would lose productivity.

Objectives:

Be able to describe the critical differences between the fungal groups Basidiomycota, Zygomycota, and Ascomycota. How are their life cycles all similar? If given a specific example be able to identify common features of that life cycle, including where there are the shifts in ploidy, shared with other fungi. Be able to describe how the structures in which they each create spores differ between fungal groups. How are the life cycles of these fungi similar to that of other eukaryotes, and how do they differ from that of animals?

What are the distinctive features of the Kingdom Fungi that separates it from other eukaryotic kingdoms? What are three different ways in which fungi might acquire food?

To a geneticist what are the advantages of: Examining a trait of a haploid stage of the life cycle compared to working with a diploid stage? The retention of the products of meiosis in an ordered arrangement versus having these products be released? The ability to grow single-celled eukaryotic organisms and make artificial chromosomes that function within them, compared to working with multicellular species for which no artificial chromosomes are readily available?

For review see self-quiz questions #1, 4-6 of this chapter.

Needed overheads and items:

Fig. 31.2	Basidiomycete, and mycelia
Fig. 31.3,	Hyphae
Fig. 31.4,	parasites and predation
Fig. 31.14	(Campbell and Reece, 2002) mycorrhizae
Fig. 31.23	lichen
Fig. 31.24	lichen
Fig. 31.5	fungal life cycle
Fig. 31.11	Phylogeny of fungi
Fig. 31.13	Zygomycota life cycle, bread mold
Fig. 31.13	Zygosporangium
Fig. 31.14	*Pilobolus* aiming sporangia
Fig. 31.20	Fairy ring
Fig. 31.18	Basidiomycetes
Fig. 31.19	Basidiomycota life cycle
Fig. 28.15	(Purves etal., 1998) basidiomycota3.jpg, basidia
Fig. 31.16	Ascomycetes
Fig. 31.17	(Campbell and Reece, 2005) Ascomycota life cycle
Fig. 31.10,	(Campbell and Reece, 2005) Asci in perithecium

Gray Black Cross no crossing over.jpg (Glase 2006; fig. 11.5)
Gray Black Cross with crossing over. jpg (Glase 2006; fig. 11.4)

Fig. 31.26	*Penicillium* sp.
Fig. 31.25	rusts on plants
Fig. 31.18	(Campbell and Reece, 2002) mycorrhizae
Fig. 31.7	yeast
Pg. 652,	(Campbell et al., 2008) features of fungal groups

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Fung. Chapter 31. Pages 636-653. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-Biology, 7th edition, Figures 31.10, 31.17. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-Biology, 6th edition, Figures 31.14, 31.18. Benjamin Cummings Press. San Francisco, CA.

Glase J.C- 2006-A Study of the Genetics of *Sordaria fimicola*- Figures 11.4, 11.5. Investigative Biology: A Laboratory Text, 2006-07. K-C. Chen and P.R. Ecklunds editors. BIO 103-104, Cornell University. Ithaca, N.Y.

Purves W.K., G.H. Orians, H.C. Heller, D. Sadava- 1998-Life. The Science of Biology. 5th edition. Fig. 28.15. Sinauer Associates. Sunderland, MA.

Related issues:

For a nice overview of the fungi and what they can do see:

> Griffin D.H- 1994-Introduction to the fungi- Chapter 1, pgs. 1-22, in <u>Fungal Physiology</u>- 2nd edition. Wiley-Liss Press, N.Y.

Fungi associate with various algal species to form **lichen**. They also form other symbiotic associations, including **mycorrhizae** with plants, or are grown by ants for food.

> Aanen D.K., H.H. de Fine Licht, A.J.M. Debets, N.A.G. Kerstes, R.F. Haekstra, J.J. Boomsma- 2009-High symbiont relatedness stabilizes mutualistic cooporation in fungus-growing termites- Science 326: (#5956, 11/20) 1103-1106
>
> Bidartondo M.I., A.M. Kretzer, E.M. Pine, T.D. Bruns- 2000-High root concentration and uneven ectomycorrhizal diversity near *Sarcodes sanguinea* (Ericaceae): A cheater that stimulates its victums?- American Journal of Botany 87: (#12) 1783-1788
>
> Gadkar V., R. David-Schwartz, T. Kunik, Y. Kapulnik- 2001-Arbuscular mycorrhizal fungal colonization. Factors involved in host recognition- Plant Physiology 127: (#4) 1493-1499
>
> Hammer S- 2000-Meristem growth dynamics and branching patterns in the Cladoniaceae- American Journal of Botany 87: (#1) 33-47
>
> Kernan M.J., A.F. Finocchio- 1983-A new discomycete associated with the roots of *Monotropa uniflora* (Ericaceae)- Mycologia 75: (#5) 916-920
>
> Maherali H., J.N. Klironomos- 2007-Influence of phylogeny on fungal community assembly and ecosystem functioning- Science 316: (#5832, 6/22) 1746-1748
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> Márquez L.M., R.S. Redman, R.J. Rodriquez, M.J. Roossinck- 2007- A virus in a fungus in a plant: Three-way symbiosis required for thermal tolerance- Science 315: (1/26) 513-515
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> McCormick M.K., K.L. Gross, R.A. Smith- 2001-*Danthonia spicata* (Poaceae) and *Atkinsonella hypoxylon* (Balansiae): Environmental dependence of a symbiosis- American Journal of Botany 88: (#5) 903-909
>
> Sanders W.B-2002-*In situ* development of the foliicolous lichen Phylophiale (Trichotheliaceae) from propagule germination to propagule production- American Journal of Botany 89: (#1) 1741-1746

This article describes a fungus that infects plant flowers and helps the plant to attract **pollinators**. A rather complex symbiosis.

> Sakai S., M. Kato, H. Nagamasu- 2000-*Artocarpus* (Moraceae) gall midge pollination mutualism mediated by a male-flower parasitic fungus- American Journal of Botany 87: (#3) 440-445

For more on **truffles**...

Bohannon J- 2009-Rooting around the truffle genome- Science 323: (#5917, 2/20) 1006-1007

Trappe J.M., A.W. Claridge- 2010-The hidden life of truffles- Scientific American 302: (#4, April) 78-84

Some reports suggest that a **fungal infection** may be associated with the global collapse of **amphibian** populations.

Pennisi E- 2009-Life and death play out on the skins of frogs- Science 326: (#5952, 10/23) 507-508

Voyles J., S. Young, L. Berger, C. Campbell, W.F. Voyles, A. Dinudom, D. Cook, R. Webb, R.A. Alford, L.F. Skerratt, R. Speare- 2009-Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian decline- Science 326: (#5952, 10/23) 582-585

Yeast has been found to lack any **RNA interference** system (RNAi). Here are some articles that examines what is needed to put an RNAi system into yeast that did not have it before.

Moazed D- 2009-Rejoice - RNAi for yeast- Science 326: (#5952, 10/23) 533-534

Drinnenberg I.A., D.E. Weinberg, K.T. Xie, J.P. Mower, K.H. Wolfe, G.R. Fink, D.P. Bartel- 2009-RNAi in budding yeast- Science 326: (#5952, 10/23) 544-550

Here are some articles relating to **fungal genetics**. How we can alter their genetics for our benefit (like making beer!). Also the genomes of several fungi species have been sequenced, and errors during cell division that lead to genetic diversity are amongst the issues that are being studied.

Alper H., J. Moxley, E. Nevoigt, G.R. Fink, G. Stephanopoulos- 2006-Engineering yeast transcription machinery for improved ethanol tolerance and production- Science 314: (12/8) 1565-1568

Cuomo C.A., U. Güldener, J-R. Vu, F. Trail, G.G. Turgeon, A. DiPietro, J.D. Walton, L-J. Ma, S.E. Baker, M. Rep, G. Adam, J. Antoniw, T. Baldwin, S. Calvo, Y-L. Chang, D. DeCaprio, L.R. Gale, S. Gnerre, R.S. Goswami, K. Hammond-Kosack, L.J. Harris, K. Hilburn, J.C. Kennell, S. Kroken, J.K. Magnuson, G. Mannhaupt, E. Mauceli, H-W. Mewes, R. Mitterhauser, G. Muchlbauer, M. Münsterkõtter, D. Nelson, K. O'Donnell, T. Ouellet, W. Qi, H. Quesneville, M.I.G. Rancero, K-Y. Seong, I.V. Tetko, M. Urban, C. Waalwijk, T.J. Ward, J. Yao, B.W. Birrea, H.C. Kistler- 2007-The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization- Science 317: (#5843, 9/7) 1400-1402

Torres E.M., T. Sokolsky, C.M. Tucker, L.Y. Chan, M. Boselli, M.J. Dunham, A. Amon- 2007-Effects of aneuploidy on cellular physiology and cell division in haploid yeast- Science 317: (#5840, 8/17) 916-924

Our models of the relationships (i.e. **phylogeny**) between different fungal groups has been changed quite a bit over the past few decades.

Lutzoni F., F. Kauff, C.J. Cox, D. McLaughlin, G. Celio, B. Dentinger, M. Padamsee, D. Hibbett, T.Y. James, E. Baloch, M. Grube, V. Reeb, B. Hofstetter, C. Schoch, A.E. Arnold, J. Miadlikowska, J. Spatafora, D. Johnson, S. Hambleton, M. Crockett, R. Shoemaker, G-H. Sung, R. Lücking, T. Lumbsch, K. O'Donnell, M. Binder, P. Diederick, D. Ertz, C. Gueidan, K. Hansen, R.C. Harris, K. Hosaka, Y-W. Lim, B. Matheny, H. Nishida, D. Pfister, J. Rogers, A. Rossman, I. Schmitt, H. Sipman, J. Stone, J. Sugiyama, R. Yahr, R. Vilgalys- 2004-Assembling the fungal tree of life: Progress, classification, and evolution of subcellular traits- American Journal of Botany 91: (10) 1446-1480

Spatafora J.W., B. Volkmann-Kohlmeyer, J. Kohlmeyer- 1998-Independent terrestrial origins of the Halosphaeriales (marine ascomycota)- American Journal of Botany 85: (#11) 1569-1580

Fungi, just like other multicelled organisms, must defend against pathogens. Here is an article about the **innate immune defenses** found in one fungus.

Schneider T., T. Kruse, R. Wimmer, I. Wiedemann, V. Sass, U. Pag, A. Jansen, A.K. Nielsen, P.H. Mygind, D.S. Raventós, S. Neve, B. Ravin, A.M.J.J. Bonvin, L. De Maria, A.S. Andersen, L.K. Gammelgaard, H-G. Sahl, H.H. Kristensen- 2010- Plectasin, a fungal defensin, targets the bacterial cell wall precursor lipid II- Science 328: (#5982, 5/28) 1168-1172

BIO 108   2010
Day 3, Lecture 7, Title: DNA as Genetic Material.
Text Readings: Campbell et al. (2008), Pgs. 86-89, 305-310.

Topics to cover:

      Early Views of Genetic Factors
      Evidence Identify the Genetic Material
            Griffith
            Avery, McCarty and MacLeod
            Hershey and Chase
            Chargaff
      DNA Structure
            Pauling's work
            Franklin
      Summary

Early Views of Genetic Factors
What Darwin didn't know...
Early views of heritable molecules.
      A few old models of inheritance; pangenesis?
      Early cell division staining: argued over whether this showed truth or artifacts?
Genetic factors must be segregated so mitosis, meiosis patterns make sense
      This follows from Sutton's work, and his chromosomal theory of inheritance
            Recall that Morgan confirmed that genetic factors moved with chromosomes
      cytosolic segregation, cytokinesis, allows segregation of DNA in other organelles...
In 1920s-1930s a "hot topic" in biology was proteins,
      many isolations and characterizations of enzyme activities being done
      Note, chromosomes are made of chromatin: both DNA and proteins present
            DNA was thought to be an unvarying polymer, proteins known to vary
            DNA thought to be a phosphate storage compound?
      so it was natural at the time to assume that proteins could carrying genetic information
            the proteins in the chromatin were thought to be the genes...

Evidence Identifing the Genetic Material
      Will review the major experiments covered in the text...
            Students should learn these experiments

      Griffith (1928)
Transforming factors and transformation
      bacteria (*Streptococcus pneumoniae*) may take up genetic factors from media
Fig. 16.2, Griffith's transformation expt.
Found that some item, taken up from dead cells altered the phenotype of live cells

this factor was heritable, suggesting that it was a heritable genetic factor
        Transformation is the uptake and integration of a genetic factor from the medium...
This gave a means to fractionate this mix from dead cells, and isolate the specific factor?

Avery, McCarty and MacLeod (1944)
Started with material from Griffith's work. A crude mix of materials from dead cells...
Avery purified the mix into distinct fractions of types of organic material
Then McCarty and MacLeod did experiments using the purified fractions
        Fig 5.4, (Hartwell etal, 2000) Avery.jpg
        to some of the fractions applied protease, RNAase, DNAase, etc...
                this degraded/removed selected types of organic matter
                the genetic material would be destroyed and transformation ability lost
only when DNA was not degraded did transformation occur,
        concluded DNA was likely to be the genetic material
Critics argued that some proteins could be resistant to degradation
        or that DNA helps stabilize the proteins, in chromatin, to allow transformation
        this would give DNA a role as a framework and protector and the item
                to which proteinaceous genetic factors were bound?
So this was taken as evidence, not proof, that DNA was genetic material

Hershey and Chase (1952)
Isotopic labeling possible after WWII
Fig. 16.3, bacteriophage
Fig. 19.1, Phage attacking *E. coli*
        Viruses of bacteria, easy to grow bacteria, easy to infect them with bacteriophage
        a nice experimental system
What in viruses codes for the production of more viruses? What is the genetic material?
        knew viruses contained both proteins (viral capsid) and Nucleic Acid polymers
Fig. 16.4, Hershey-Chase expt.
        note use of ^{32}P versus ^{35}S to label various parts of phages
Looked to see which isotope got into bacteria upon infection
        Most ^{32}P got in, very little (but some) ^{35}S got into the bacteria
Hershey and Chase concluded that this supported nucleic acid polymer as the most
                likely genetic material.
Critics argued that only a few critical proteins need enter to carry genetic info...
Again, evidence, but not proof

Chargaff (1947 on)

        Fig. 16.5, DNA bases and backbone

problem with DNA is it did not seem to vary... A mundane polymer

        if it does not vary it could not carry genetic information...?

Chargaff was an organic chemist who studied nucleic acid polymers, including DNA

        (Today this would be similar to someone studying the structure of starch...?)

Table 5.1, (Hartwell etal, 2000) Chargaff.jpg

Chargaff's rule: proportion of A = T, of C = G

        but this only limited the structure, did not suggest how info was carried...

        this does not show how DNA varied between species

Chargaff found variation of DNA from one species to another, so there is a difference

        The variation is seen in the proportions of C+G pairs used versus A+T pairs.

Some saw this as more evidence of the possible importance of DNA

        Others viewed it an interesting correlation,... but....

        different species also have different proteins with different amino acid ratios?

            so still some critics arguing at this time for proteins as possible genes...

DNA structure

Knowing a molecule's structure is immensely helpful if wish to understand a molecule's functions

So in late 1940-early 1950s there was wide interest in determining the structure of DNA

Pauling (1953)   (see paper, Pauling and Corey, 1953)

Fig. 16.7, DNA structure

Fig. 5.01a, (Campbell and Reece, 2002) LinusPauling

A race, Linus Pauling published one paper, but based on poor data from poor crystals
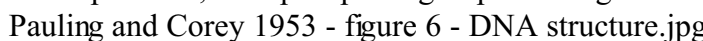
Fig. 5.25, X-ray crystallography

        Use of this technique. Depends on good purification of sample and crystals of it

        Pauling used this technique to determine protein structure (alpha helix)

            attempted to determine DNA structure, but sample and crystals not good

        Proposed a triple helix, with phosphate groups sticking in and bases out

            Pauling and Corey 1953 - figure 6 - DNA structure.jpg

Franklin (etal. 1953a)

Rosalind Franklin's X-ray diffraction data

        knew already that plane of bases perpendicular to ring of sugar

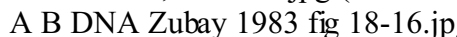   Worked in same lab as Maurice Wilkins, both as researchers (Wilkins, 2003)

        Rosalind Franklin portrait.jpg (Maddox, 2002)

            Very nice book telling her story.... (Maddox, 2002)

        Fig. 16.6, Franklin

What she did: DNA forms, A and B.jpg (Maddox, 2002)

          A B DNA Zubay 1983 fig 18-16.jpg

She determined that there were two forms of DNA in crystals (Franklin etal. 1953c)

          This matters since, how it is crystallized alters the data obtained

X ray crystallography of A form of DNA.jpg (Watson, 1968, pg. 73)
X ray crystallography of B form of DNA.jpg (Watson, 1968, pg. 168)
Franklin was assigned the B DNA form to study. Wilkins got the A DNA form.
(note that other helix forms of DNA exist such as C and Z-DNA, left handed helix)
Z DNA Zubay 1983 fig 18-17.jpg
She determined that phosphates face outward, with bases inward
Fig. 16.7, DNA structure
Discovered the antiparallel nature of the two strands of DNA
Calculated the unequal twist of the helix, and the size of the basic BP unit.
Determined the width of the dsDNA helix, which limited base pairing
Wilkins did an analysis of her data showing it was consistent with a helix
(Wilkins et al. 1953)
Though Watson (1968) seems to claim to have done some of the above...
her data were used without her knowledge or permission, before it was published
she assumed Watson and Crick's work was pure theory
died (1958) before Nobel prize awarded (1962) to survivors, not her
Fig. 16UN-298, PurinePyrimidine
Fig. 16.1, Watson and Crick
Complementary base pairing implied replication and gene expression roles...
Structural information was interesting, but still not proof,
functional questions still remained
but with structure, DNA was now accepted as the genetic material

Summary

DNA does what?
Information carrying: sometimes DNA can act as an enzyme

Other information carrying molecules have been demonstrated *in vitro*, and a few in life,
so DNA does NOT need to be only genetic material
Biotic forms
RNA: retroviruses, HIV
Proteins: prions, centrioles?

The text (Campbell et al. 2008, pg 305, first column, second paragraph, first sentence) suggests that only nucleic acids are known "... to direct their own replication from monomers.."
This is just not true. Other molecules can guide their own replication.
For examples of synthetic replicative molecules note examples from Rebek (1994)

Objectives:

Be able to describe the studies done by 1) Griffith, 2) Avery and MacLeod and McCarty, 3) Hershey and Chase, 4) Franklin and Wilkins and Watson and Crick, and 5) Chargaff. What evidence did each study provide in support of DNA as a molecule able to carry genetic information? Be able to describe the structure of DNA. Is DNA the sole known carrier of genetic material? What is one example where RNA carries information?

What were some of the major reservations about DNA as genetic material? What alternative was proposed to DNA? Based just on what Griffith found from his transformation experiment describe how it would be possible for the transforming factor to have been DNA or RNA or protein.

What made ^{32}P and ^{35}S good isotopic markers for use by Hershey and Chase? What types of organic molecules does each tend to mark? Are these elements only found in those classes of molecules? What would have been the problem if ^{14}C or ^{15}N had been used as isotopic markers?

What is X ray crystallography, what must one have to do it properly, and what information can be obtained using it?

For review, see self-quiz questions #1 and 5 at the end of chapter 16.

Needed overheads and items:

     Fig. 16.2, Griffith's transformation expt.
     Fig. 5.4, (Hartwell etal, 2000) Avery.jpg
     Fig. 16.3, bacteriophage
     Fig. 19.1, Phage attacking *E. coli*
     Fig. 16.4, Hershey-Chase expt.
     Fig. 16.5, DNA bases and backbone
     Table 5.1, (Hartwell etal, 2000) Chargaff.jpg
     Fig. 16.7, DNA structure
     Fig. 5.01a, (Campbell and Reece, 2002) Linus Pauling
     Fig. 5.25, X-ray crystalography
     Pauling and Corey 1953 - figure 6 - DNA structure.jpg
     Rosalind Franklin portrait.jpg (Maddox, 2002)
     Fig. 16.6, Franklin and X ray data
     DNA forms, A and B.jpg (Maddox, 2002)
     A B DNA Zubay 1983 fig 18-16.jpg
     X ray crystallography of A form of DNA.jpg (Watson, 1968, pg. 73)
     X ray crystallography of B form of DNA.jpg (Watson, 1968, pg. 168)
     Z DNA Zubay 1983 fig 18-17.jpg
     Fig. 16.7, DNA structure
     Fig. 16UN-298, Purine Pyrimidie
     Fig. 16.01, Watson Crick

Handout:
     (Handout - Lecture 7.stm)
     Table 5.1, (Hartwell etal, 2000) Chargaff.jpg
     Fig. 5.4, (Hartwell etal, 2000) Avery.jpg

(Bring in Book, Maddox, 2002)

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-<u>Biology</u>. Eighth edition. Pgs. 86-89, 305-310. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Fig. 5.01. Benjamin Cummings Press. San Francisco, CA.

Franklin R.E., R.G. Gosling- 1953a-Molecular configuration in sodium thymonucleate- Nature 171: (#4356, Apr. 25) 740-741

Franklin R.E., R.G. Gosling- 1953c-The structure of sodium thymonucleate fibers. I. The influence of water content- Acta Crystallographica- 6: 673-677

Franklin R.E., R.G. Gosling- 1953d-The structure of sodium thymonucleate fibres. II. The cylindrically symmetrical Patterson function- Acta Crystallographica 6: 678-685

Hartwell L., L. Hood, M.L. Goldberg, A.E. Reynolds, L.M. Silver, R.C. Veres- 2000-<u>Genetics. From genes to genomes</u>. Figures 5.4, and Table 5.1. McGraw-Hill Press. Boston, MA.

Maddox B- 2002-<u>Rosalind Franklin: The dark lady of DNA</u>. 380 pgs. Harper Collins Publishers, N.Y., N.Y.

Pauling L., R.B. Corey- 1953-A proposed structure for the nucleic acids- Proceedings of the National Academy of Sciences (USA) 39: 84-97

Rebek J. (jr)- 1994-Synthetic self-replicating molecules- Scientific American 271: (#1) 48-55

Watson J.D- 1968-<u>The Double Helix. A personal account of the discovery of the structure of DNA</u>. 237 pages. Atheneum Press, N.Y.

Watson J.D., F.H.C. Crick- 1953-Molecular structure of nucleic acids- Nature (#4356, 4/25) 737-738

Wilkins M- 2003-<u>The Third Man of the Double Helix</u>. Oxford University Press. Oxford, U.K. 274 pgs.

Wilkins M.H.F., A.R. Stokes, H.R. Wilson- 1953-Molecular structure of deoxypentose nucleic acids- Nature 171: (April 25) 738-740

Zubay G- 1983-Structure of nucleic acids and nucleoproteins. Chapter 18. Figures 18.16, 18.17, in <u>Biochemistry</u>. Addison-Wesley Publishing Co. Reading, MA.

Related issues:

For more on the **structural variations of DNA**, including the A, B, and Z forms, see:

Franklin R.E., R.G. Gosling- 1955-The structure of sodium thymonucleate fibers. III. The three-dimensional Patterson function- Acta Crystallographica 8: 151-156

Franklin R.E., R.G. Gosling- 1953b-Evidence for 2-chain helix in crystalline structure of sodium deoxyribonucleate- Nature 172: (#4369, 7/25) 156-157

Nakata M., G. Zanchetta, B.D. Chapman, C.D. Jones, J.D. Cross, R. Pindak, T. Belini, N.A. Clark- 2007-End-to-end stacking and liquid crystal condensation of 6-to-20 base pair DNA duplexes- Science 318: (#5854, 11/23) 1276-1279

Wilkins M.H.F., R.G. Gosling, W.E. Seeds- 1951-Physical studies of nucleic acid- Nature 167: (#4254, May 12) 759-760

Zubay G- 1983-Structure of nucleic acids and nucleoproteins. Chapter 18. Pgs. 665-685, in Biochemistry. Addison-Wesley Publishing Co. Reading, MA.

DNA is not the only possible **genetic material**. Here are some articles that point out other possible inherited items, including **RNAs**.

Bean H.D., F.A.L. Anet, I.R. Gold, N.V. Hud- 2006-Glyoxylate as a backbone linkage for a prebiotic ancestor of RNA- Origins of life and evolution of biospheres- 36: 39-63

Bloch D.P- 1988-Cybernetic origins of replication- Origins of life and evolution of the biosphere- 18: 87-96

Deamer D.W., G.R. Fleischaker- 1994-Bioinformational Molecules- Section V, pgs 337-340 in Origin of Life: The central concepts- Jones and Bartlett publisher. Boston, MA.

Eigen M. J. McCaskill, P. Schuster- 1988-Molecular quasi-species- Journal of Physical Chemistry 92: 6881-6891

Gibbs W.W- 2003-The unseen genome-Scientific American 289: (#6) 106-113

Minkel J.R- 2005-RNA to the rescue: Novel inheritance patterns violate Mendel's law- Scientific American 292; (#6, June) 20-22

Robertson M.P., W.G. Scott- 2007-The structural basis of ribozyme-catalyzed RNA assembly- Science 315: (3/16) 1549-1553

Shapiro R- 2007-A simpler origin of life- Scientific American 296: (#6, June) 46-53

DNA does not have to be just genetic material, there have been DNAs that were found *in vitro* to have enzymatic activities (i.e. a **deoxyribozyme**).

Santoro S.W., G.F. Joyce- 1997-A general purpose RNA-cleaving DNA enzyme- Proceedings of the National Academy of Sciences (USA) 94: 4262-4266

The process of **transformation** is of great interest since it has been noted to produce genetic variation, and often to be associated with cancers. Here are some studies that make use of, or tell about, transformation.

Chilton M-D-2001-*Agrobacterium*. A memoir- Plant Physiology 125: 9-14

Hartwell L., L. Hood, M.L. Goldberg, A.E. Reynolds, L.M. Silver, R.C. Veres- 2000-The prokaryotic chromosome: Genetic analysis in bacteria- Chapter 13, pgs. 461-500, in, Genetics: From Genes to Genomes- McGraw Hill. Boston, MA.

Leone A., U. Flatow, C.R. King, M.A. Sandeen, I.M.K. Margulies, L.A. Liotta, P.S. Steeg- 1991-Reduced tumor incidence, metastatic potential, and cytokine responsiveness of *nm23*-transfected melanoma cells- Cell 65: 25-35

Mayr C., M.T. Hemann, D.P. Bartel- 2007-Disrupting the pairing between *let*-7 and Hmga2 enhances oncogenic transformation- Science 315: (3/16) 1576-1579

Sommer M.O.A., G. Dantas, G.M. Church- 2009-Functional characterization of the antibiotic resistance reservoir in the human microflora- Science 325: (#5944, 8/28) 1128-1131

BIO 108   2010
Day 3, Lecture #8, Title: DNA Replication.
Text Readings: Campbell et al. (2008), pgs. 311-319.

Topics to cover:

> **Models of DNA Replication**
> > **Meselson and Stahl Experiment**
> **Molecular Process of Replication**
> > **Origins of Replication**
> > **Replication Bubbles**
> > **Enzymes, Proteins, and other Needed Items**
> > **Telomeres and Ends of Linear Chromosomes**

Models of DNA Replication
Watson and Crick's proposal of replication
> Fig. 16.9, basic Watson/Crick replication
> A purine (A or G) and a pyrimidine (T or C) as a pair gives same width
> Fig. 16.8, base pairing
> they match each other with H-bonds
> > A good use of weak bonds (H-bonds), as allows access...?
> > Note, complementation is not copying... so DNA replication is not copying...
> But this does not answer everything, other questions include:
> > In what order does it replicate along the DNA?
> > How is replication initiated and controlled?
> > How is replication of ends of a linear chromosome achieved?
Three variations on above
> Fig. 16.10, alternative models of replication
> Conservative, semi-conservative, dispersed models of replication
> > (in reality all of these are done in specific cases... we are here concerned with just
> > replication of the major regions of the nuclear chromosomes during S-phase of
> > the cell cycle...)
> **Meselson and Stahl Experiment**
> Semi-conservative replication: consider experimental evidence for it
> Fig. 5.16, (Hartwell etal, 2000) Meselson_Stahl.jpg
> Fig. 16.11, Messelson-Stahl expt
> Use of ^{14}N and ^{15}N to label newly replicated DNA
> > since isolating DNA, can use a common isotope in this case
> > the proteins and other N containing molecules can be removed from the extract...
> ultra-centrifugation separates on the basis of density
> > use of a different N isotope will alter the density of the polymer
> note different predictions of densities of products for each of three models of replication
> So compared observations to these predictions, support for semi-conservative model

Molecular Process of Replication
Replication of DNA
Mainly during S phase of cell cycle, for nuclear DNA, connect to chromatids in meiosis
DNA in mitochondria and chloroplasts replicates during almost all parts of cell cycle...
Some nuclear DNA also replicates outside of S phase...
Viral replication is also very diverse...
Add to just 3' end of existing DNA, note antiparallel strands so 3' ends are not together
Fig. 16.14, adding at 3' end
Fig. 16.12, (Campbell and Reece, 2002) antiparallel strands, 3' ends
In theory it is possible to grow both ends? Both the 3' and 5'?
But it does not do it that way! In part to allow for removal of mistakes...

Origins of Replication
Fig. 16.12, origin of replication
Regions of DNA where proteins bind to start replication, these areas are not genes...
so does not start anywhere, start points are controlled
note that some bacteria replicate selected regions in it to plasmids
so the ORI on plasmids must be distinctly regulated?
note that some eukaryotic ORI sites are used first, so also regulated somehow...
Fig. 5.31, (Alberts etal, 2002) origin.jpg
Note how proteins bind, then replication begins,
note role of helicase to converst dsDNA into ssDNA,
and primase to lay down an RNA section to give a 3' end
many other proteins involved here. If not present, can not start replication
MBOTC , CD, movie 5.1, DNA helicase, showing changes in protein structure
Fig. 16.16, primer and primase
This ends with positioning the beginning of process
you would think it would start with DNA
but no, it starts with RNA, replaces this later
Primase lays down RNA complementary to the exposed ssDNA.
two types of DNA polymerases (I and III) are shown in this figure in the text

Replication Bubbles and Forks
Eventually opens into a replication bubble which has two replication forks, one at each end
Fig. 16.14, (Campbell and Reece, 2005) replication fork
note Replication Forks and orientation of DNA template strands and of new strands.
Leading and lagging strands
You would think that it would be continuous,
but no, only leading strand is continuous
Fig. 16.17, summary of replication (note error!)
this figure is not how it looks, will show real structure in a bit

this figure suggests site of synthesis of leading strand can be far from where
lagging strand is made, but that is not correct

Note Okazaki fragments, each starts with a short section of RNA
Rates of replication
in prokaryotes about 500 nucleotides/sec are laid down
in eukaryotes about 50 nucleotides/sec are laid down, so slower
must de-condense the DNA, remove histones, does more error-correction
MBOTC, CD, movie 5.4, DNA replication fork

Enzymes, Proteins, and other Needed Items (See Table 16.1)
Note names and functions of following (draw on board and list)
initiation proteins   at ORI
Helicase   Topoisomerases to relieve twist...
Single-stranded binding proteins (ssb proteins)
Primase (an RNA polymerase)
DNA polymerases (various types, humans have 10 known types...)
III in fork, I replaces RNA primer, II does repair
DNA ligase
For RNA/DNA need monomers:
ATP, CTP, UTP, GTP, and dATP, dCTP, dTTP, dGTP
Later on can modify the DNA, via acetylation or methylation, influencing gene expression

Telomeres and Ends of Linear Chromosomes
Ends of linear chromosomes
Fig. 16.19, end-replication problem
Gap left when RNA primer was removed so 3' end stick out further than 5' end
The dangling ssDNA will be cut off by restriction enzymes if left like this
That would shorten the DNA each time it replicates?
Fig. 16.20, telomeres
When telomerase activity is lacking, cells can only go through about 50 cell cycles
This is the situation form most of our body cells, but not for stem cells...
stem cells have active telomerase, as do cancer cells...
Telomeres are regions of DNA at ends of linear DNA
so, if you have a circular chromosome this is not a problem...
telomerase, uses RNA as template to guide formation of DNA
Fig. 16.19, (Campbell and Reece, 2002) telomerase
Fig. 5.42, (Alberts etal, 2002) telomerase.jpg
A protein-RNA complex, RNA used as template for DNA
sort of like reverse-transcription?

telomeres

Fig. 5.43, (Alberts etal, 2002) telomeres.jpg

Lays down repeated units, extending 3' end. Gives room for an RNA primer to be added

Then RNA primer then gives a 3' end for DNA polymerase to work on, later RNA is lost..

If ssDNA is lost now, that is OK because it is extra...

Ends of linear chromosomes are complex structures.

T-loops

>Fig. 5.44, (Alberts etal, 2002), T-loops.jpg

>Seen in our and some other mammalial chromosome ends

>ssDNA folds back on itself, held by proteins

>dsDNA also folded back, as heterochromatin

>also have unique proteins associated with the DNA here

>gives ends distinctive structure and protein composition at ends...

>many types of proteins and even RNAs involved in these structures.

DNA replication

>A complementing system, not a copying system. Mostly semi-conservative.

>Needs RNA as primer. Starts at ORI sites. Adds to 3' ends.

>Note table 16.1 for main enzymes involved. (Many others exist.)

>This is how single-chromatid chromosomes become double-chromatid chromosomes.

>Mainly during S phase of cell cycle

>>DNA replication at centromere may not be completed until later in cell cycle...

>>DNA replication in organelles at other times as well.

>Remember that this is not gene expression...

>>Will cover gene expression processes involved in that next lectures...

Objectives:

Be able to describe the Meselson-Stahl experiment on the pattern of DNA replication. What were they able to show, and how did the evidence they obtained enable them to disprove some of the alternate models of replication?

You should be able to describe the process of DNA replication. All of the items shown in figures 16.13-16.17, and listed in table 16.1, of your text are fair game. Be sure you know the function(s) of each item given there, and the order in which they are used during this process.

What are origins of replication? Does DNA polymerase or an RNA polymerase act earlier at this site? Why should a eukaryotic chromosome have many origins of replication? Be able to contrast replication on leading strands with that done on lagging strands.

At what point in the cell cycle does most DNA replication occur? In a eukaryotic cell which DNA is replicated at variable times and which is replicated mainly at one time in the cell cycle? How is the fact that the DNA was replicated evident in the chromosome structure observed during cell division?

What are telomeres? What role do they play and what problem do they prevent? Why would you expect to find no telomeres in the chromosomes of bacteria or of mitochondria? What enzyme is involved in the production of telomeres, and what would happen to a cell if the expression of the gene for this enzyme was turned off? Do all human cells express this gene all the time?

For review see self-quiz questions #2, 3, 4, and 6 of chapter 16.

Needed overheads and items:

Fig. 16.9, basic Watson/Crick replication
Fig. 16.8, base pairing
Fig. 16.10, alternative models of replication
Fig. 5.16, (Hartwell etal, 2000) Meselson_Stahl.jpg
Fig. 16.11, Messelson-Stahl expt
Fig. 16.14, adding at 3' end
Fig. 16.12, (Campbell and Reece, 2002) antiparallel strands, 3' ends
Fig. 16.12, origin of replication
Fig. 5.31, (Alberts etal, 2002) origin.jpg

MBOTC, CD, movie 5.1, DNA helicase, shows changes in protein structure

Fig. 16.16, primer and primase
Fig. 16.14, (Campbell and Reece, 2005) replication fork
Fig. 16.17, summary of replication (note error!)

MBOTC, CD, movie 5.4, DNA replication fork, shows real alignment

Fig. 16.19, end-replication problem
Fig. 16.20, telomeres
Fig. 16.19, (Campbell and Reece, 2002) telomerase
Fig. 5.42, (Alberts etal, 2002) telomerase.jpg
Fig. 5.44, (Alberts etal, 2002), T-loops.jpg

Handout:
Handout - lecture 8.stm
Fig. 5.31, (Alberts etal, 2002) origin.jpg
Fig. 5.43, (Alberts etal, 2002) telomeres.jpg

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002-<u>Molecular Biology of the Cell</u>. 4th edition. Figures. 5.31, 5.42, 5.43, 5.44. Garland Science Publishing. N.Y., N.Y.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-The molecular basis of inheritance. Chapter 16. Pages 311-319. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition, Fig. 16.14. Benjamin Cummings Press. San Francisco, CA.

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Hartwell L., L. Hood, M.L. Goldberg, A.E. Reynolds, L.M. Silver, R.C. Veres- 2000-<u>Genetics. From Genes to Genome</u>. Fig. 5.15. McGraw Hill Publishers. Boston, M.A.

Related issues:

DNA polymerases were discovered by **Arthur Kornberg**, who died in 2007. Here is an article that reviews his life's work, and the citation of one of his studies on a DNA polymerase.

> Berg P., I.R. Lehman- 2007-Arthur Kornberg (1918-2007)- Science 318: (#5856, 12/7)
> 1564
> Josse J., A.D. Kaiser, A. Kornberg- 1961-Enzymatic synthesis of deoxyribonucleic acid.
> VIII. Frequencies of nearest neighbor base sequences in deoxyribonucleic
> acid- Journal of Biological Chemistry 236: (#3) 864-875

We like to think that replication of DNA is done during S phase of the **cell cycle**. Here is a report of replication forks that are active during mitosis!

> Weinert T- 2007-What a cell should know (But may not)- Science 315: (3/9) 1374-
> 1375

This article notes that the **replication forks rotate** as they move down the DNA. This causes the two sister chromatids to end up being intertwined after replication.

> Murray A.W., J.W. Szostak- 1985-Chromosome segregation in mitosis and meiosis-
> Annual Review of Cell Biology 1: 289-315

The **histones** of the nucleosomes close to a replication fork must be modified as the fork moved during DNA replication.

> Groth A., A. Corpet, A.J. Cook, D. Roche, J. Bartek, J. Lukas, G. Almouzni- 2007-
> Regulation of replication fork progression through histone supply and demand-
> Science 318: (#5858, 12/21) 1928-1931

Here are articles about **telomerase** and the issue of replicating of the ends of linear chromosomes and the making and functions of **telomeres**.

> de Lange T- 2009-How telomeres solve the end-protection problem- Science 326: (#5955,
> 11/13) 949-952
> Sfeir A., S. Kabir, M. van Overbeek, G.B. Celli, T. deLang- 2010-Loss of Rap1 induces
> telomere recombination in the absence of NHEJ or a DNA damage signal- Science
> 327: (#5973, 3/26) 1657-1661
> Venteicher A.S., E.B. Abreu, Z. Meng, K.E. McCain, R. M. Terns, T.D. Veenstra, M.P.
> Terns, S.E. Artandi- 2009-A human telomerase holoenzyme protein required for
> Cajal body localization and telomere synthesis- Science 323: (#5914, 1/30) 644-
> 648

For more on **nucleosome** structure and the uses of histones:

Dion M.F., T. Kaplan, M. Kim, S. Buratowski, N. Friedman, O.J. Rando- 2007- Dynamics of replication-independent histone turnover in budding yeast- Science 315: (3/9) 1405-1408

Konev A.Y., M. Tribus, S.Y. Park, V. Podhraski, C.Y. Lim, A.V. Emelyanov, E. Vershilova, V. Pirrata, J.T. Kadonaga, A. Lusser, D.V. Fyodorov- 2007-CHD1 motor protein is required for deposition of histone variant H3.3 into chromatin *in vitro*- Science 317: (#5841, 8/24) 1087-1090

For more on various aspects of **DNA replication**. Including the helicase that unwinds the DNA. On DNA polymerases. Metabolic influences on replication. The structure of the origins of replication, and other issues...

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walker- 2002-DNA replication, repair, and recombination- Chapter 5, pgs. 235-298, in Molecular Biology of the Cell. 4th edition. Garland Science, N.Y., N.Y.

Bailey S., W.K. Eliason, T.A. Steitz- 2007-Structure of hexameric DnaB helicase and its complex with a domain of DnaG primase- Science 318: (#5849, 10/19) 459-463

Burhans W.C., J.A. Huberman- 1994-DNA replication origins in animal cells: A question of context?- Science 263: 639-640

Chen Z., E.A. Odstrcil, B.P. Tu, S.L. McKnight- 2007-Restriction of DNA replication to the reductive phase of the metabolic cycle protects genome integrity- Science 316: (#5833, 6/29) 1916-1919

Cunningham-Dueber E.L., J.E. Corn, S.D. Bell, J.M. Berger- 2007-Replication origin recognition and deformation by a heterodimeric Archaeal Orc1 complex- Science 317: (#5842, 8/31) 1210-1213

Flint S.J., L.W. Enquist, V.R. Racaniello, A.M. Skalka- 2004-Genome replication strategies: DNA viruses, chapter 9, pgs. 298-339, in Principles of Virology: Molecular biology, pathogenesis, and control of animal viruses- ASM Press. Washington D.C.

Gaudier M., B.S. Schuwirth, S.L. Westcott, D.B. Wigley- 2007-Structural basis of DNA replication origin recognition by an ORC protein- Science 317: (#5842, 8/31) 1213-1216

Georgescu R.E., M. O'Donnell- 2007-Getting DNA to unwind- Science 317: (#5842, 8/31) 1181-1182

Ide S., T. Miyazaki, H. Maki, T. Kobayashi- 2010-Abundance of ribosomal RNA gene copies maintains genome integrity- Science 327: (#5966, 2/5) 693-696

Pursell Z.F., I. Isoz, E-B. Lundström, E. Johansson, T.A. Kunkel- 2007-Yeast DNA polymerase ε participates in leading-strand DNA replication- Science 317: (#5834, 7/6) 127-130

This report focuses on replication in *E. coli* and the structure of the **replisome**..

Reyes-Lamothe R., D.J. Sherratt, M.C. Leake- 2010-Stoichiometry and architecture of active DNA replication machinery in *Escherichia coli*- Science 328: (#5977, 4/23) 498-501

Replication of the DNA in the **plastids** is also of interest:

Nerozzi A.M., A.W. Coleman- 1997-Localization of plastid DNA replication on a nucleoid structure- American Journal of Botany 84: (#8) 1028-1041

Our ability to replicate DNA for our purposes has some technical challenges, and opens the possibility of **designed life**.

Baker D., G. Church, J. Collins, D. Endy, J. Jacobson, J. Keasling, P. Modrich, C. Smolke, R. Weiss- 2006-Engineering life: Building a FAR for biology- Scientific American 294: (#6) 44-51

BIO 108   2010
Day 3, Lecture #9, Title: Transcription and RNA Processing.
Text Readings: Campbell et al. (2008), pgs. 320-322, 325-336, 347, 356-358, 362-366.

Topics to cover:

> **"One-gene one-protein" Concept**
> **Central Dogma**
> **Transcription**
> **RNA Processing**
> **Mature RNA Regions**
> **Export from the Nucleus**
> **RNA Interference and Riboswitches**
> **Summary**

"One-gene one-protein" Concept
Beadle and Tatum's expt.
> Fig. 17.2, Beadle and Tatum's expt
> review Arg (arginine) biosynthetic pathway, an amino acid that must be made.
> Can get mutants, grow with Arg present and mutate via UV exposure or other means.
> genetic blocks, and replacement, or "feeding", experiments done
> > block results in accumulation of intermediated, leak out and "feed" other mutants
> > "feeding" defined as ability to complete the pathway and make end product
> so changes are in units, one-gene coding for one-enzyme was the original concept
> but enzymes have many subunits, quaternary structure
> So concept was altered to one-gene one-polypeptide, with each as subunit in protein
> But since product can be a protein subunit or just a molecule of RNA,
> > we will see exceptions to even this generalization...

Central Dogma
Now covering processes of gene expression
> First general processes, then tomorrow will do more on control options
> Expression of genes involves all processes up to actual phenotype of organism
Note that this is NOT DNA replication! That is not a means of gene expression.
Central Dogma (from Francis Crick) and information flow
> During gene expression information flows from DNA to RNA to protein
> > > (exceptions abound to this....i.e. telomerase...)
> > exceptions are for DNA to RNA, I know of no exceptions of RNA to protein...
> > Note: DNA is NOT converted into RNA, RNA is NOT converted into protein.
> Define transcription and translation, as just two of many stages of gene expression
> > Fig. 17.3, transcription/translation overview
> Note also that this is complementation, not copying.

1

In doing gene expression, first need access to the DNA so can use it to guide transcription
    need to expose area (promoter and control regions) of a gene so that it can be expressed
        DNA with the gene must be accessible, not tightly condensed
        Fig. 18.7, histone modifications
        chromatin is often tightly condensed,
            and genes in such DNA can not be expressed
            Changes in condensation are Pre-Transcriptional controls

Transcription
    Will cover this process, and in later lectures will cover some ways it is regulated.
    three major substages
        Fig. 17.7, stages of transcription
        initiation of transcription
        elongation of RNA being formed by transcription
        termination of transcription

<u>Major items needed</u>
    RNA Polymerase (several types exist) has ability to make RNA polymers
    RNA monomers, ATP, CTP, UTP, GTP
    Lots of other proteins involved in regulation
        transcription factors, termination factors, etc... even RNAs can influence it...

<u>Initiation</u>
    Fig. 17.8, initiation of transcription
        promoter region, where RNA polymerase must bind
    Fig. 19.10, (Campbell and Reece, 2002)   DNA Binding Domains
        transcription factors bind and form an initiation complex
    Fig. 17.7, Transcription stages
            this complex helps RNA polymerase binding
        opening of DNA base pairs must occur, exposing ssDNA
        RNA production begins, using DNA as a template to guide RNA production
        Not a random process, so promoter must be recognized by RNA polymerase
            and this recognition can be controlled.
        Note, parts of the promoter are NOT transcribed,
            and beginning of the RNA made need not code for any amino acids...

<u>Elongation</u>
    The transition to this stage is often a point of control
    Fig. 17.7b Transcription elongation stage
    Fig. 6.8, (Alberts etal, 2002) transcription_elongation.jpg
    RNA added at 3' end, so just like DNA this RNA is made "downhill" 5' to 3'
    ssDNA strands become dsDNA again as RNA polymerase passes...

<u>Termination</u>
    A region of DNA codes for RNA with sequence coding for the end of transcription
        in some cases it may make a hairpin loop in the RNA...
    this is essential, otherwise it would march down chromosome and waste lots of energy...
    Fig. 17.7, Transcription stages
    RNA polymerase comes off of DNA

this produces RNA called a Primary transcript.

NOTE: DNA is NOT "converted into" RNA, nor is the RNA a "copy" of DNA.

Information in the DNA is used to guide formation of a complementary RNA.

Types of RNA produced

RNA polymerase II will mainly make mRNAs

RNA polymerase I will mainly make rRNAs

RNA Polymerase III will mainly make tRNAs and rRNAs

and many other types of RNA exist that are made by these enzymes.....

Revisit, one-gene one-polypeptide concept:

note then that only the genes coding for mRNAs go on to code for proteins,

yet those regions coding for tRNAs and rRNAs are also called genes.

So Beadle and Tatum's rule is too limited. Genes code for RNA as well as polypeptides

Not all RNA will be used to guide formation of proteins.

So generalization is better as: one-gene one-gene product (polypeptide or RNA)?

RNA Processing

Focus on what happens to mRNA primary transcript in eukaryotes

(some processing also done in rare cases in prokaryotes and in organelles...)

primary transcript is in nucleus, how is it altered?

Fig. 17.10, RNA splicing

note additions to primary transcript

5' cap, a modified guanosine attached in unusual manner

this is often attached during transcription

Poly-A tail at 3' end   50-100s of As added

Note: Neither the cap nor tail is coded for in DNA!!

so not all parts of RNA are coded for by DNA...

This lets cell know this is mRNA and self, so it is protected from degradation

Splice out introns

Fig. 17.12, exons and domains in proteins.

exons and introns, RNA splicing

define introns and exons, relate back to gene

Exons: regions that will be EXpressed.

Introns: regions that are INserted and will be cut out.

need to splice out introns and fuse exons.

Fig. 17.11, splicing, snRNPs

small nuclear ribonuclear proteins/particles do the splicing

snRNPs, must recognize the sites to cut and splice

structure it forms is called a spliceosome...

catalytic RNA in it, another example of ribozymes

splices out introns

Fig. 18.11, alternate RNA splicing

Alternate splicing: Splicing can be done in several ways....

Can result in several types of mature mRNAs

Revisit one-gene one-gene product concept...

     Here see one gene can produce many types of mature mRNAs, depending on splicing

     One gene in fruit flies found to code for over 14000 versions of proteins!

     Perhaps concept is better as either:

          One-gene one primary transcript?   (even this has exceptions....)

          Or: One-gene all possible gene-products based on it?

          Or: A gene gets expressed and leads to the production of product(s)?

     Note: This "concept" is flexible, it is a steriotype, but still a useful generalization

          but I raise the variations to show how many concepts can turn out to be limited

     If you go on in biology you will have to learn to play with the concepts in this way!

Mature RNA Regions

     Have now made a mature RNA product, still in the nucleus

     What are the regions of it, and what might they do?

          Fig. 17.9, mature mRNA

     Note features:

          5' cap, a modified guanosine, linked through 3 Ps! May later help ribosome bind

          Poly A tail, also helps with ribosome binding, and relates to stability of the RNA

               some RNAs are stored for days, months, before used...

          leader sequence, non coding, UnTranslated Region (UTR) at 5' end

               binds ribosome, and can be bound by proteins or RNAs

               can fold into dsRNA shapes, and be sensed by proteins...

               these folds can also form binding sites, called riboswitches

          trailer with termination signal to stop transcription, non coding

               can also form folded structures and influence the use of this mRNA

          coding region, start and stop codons, for use to guide translation

               This region holds information used later to guide protein production

          So most of a mature mRNA may be non-coding

Export from the Nucleus

Export from nucleus

     want only properly processed mature mRNAs to be allowed out via nuclear pores.

     only certain RNAs are exported, many stay in nucleus

          most RNA made is degraded in nucleus

     Fig. 6.10 nucleus

     Nuclear pores sense caps and tails, may also sense proteins bound to the RNA

RNA Interference and Riboswitches

RNA interference

        This occurs in the cytosol, can bind and degrade mature exported mRNA there

        Fig. 18.13, microRNA

        miRNA, microRNA, a short section of ssRNA (Ho 2004)

        This small ssRNA is complementary to a part of mature mRNA, so base pairs with it

                This produces a bit of dsRNA, which is sensed by proteins

                Dicer cuts it up into small single-stranded RNAs.

        Other proteins, splicer, bind miRNAs and use them to target complementary RNA

        Proteins in cytosol sense dsRNA and degraded it

                So after all of that work to make mRNA, it may be destroyed in the cytosol!

        small miRNAs can also enter nucleus and have effects there (Hwang et al., 2007)

        Cells use this system to regulate their gene expression

                But also use it to protect themselves from many viruses,

                        many have dsRNA as their genetic material, this will destroy it.

        This system is found in both eukaryotes, and in prokaryotes

        we often use this system to alter gene expression to study their products functions.

Riboswitches

        The 5'-UTR region of an mRNA acts early in transcription to form a binding site

        This is able to bind specificly, its binding then changes its shape

                This alters its being bound by the capping enzyme, promoting the 5' cap addition

        Other RNAs have such 3D folds, bind other things, and that makes them diverse in uses

        So riboswitches are binding sites made by RNA,

                analogous to binding sites made by polypeptides

                So any function you can think of a protein doing might be possible by an RNA!

Summary

Mutation may cause mRNA not to be exported

        so enzymes are not always made

        this is the basis for Beadle and Tatum experiment

        but their generalization of one-gene one-protein is now seen as too narrow?

        This is a nice example of model-modification in science...

Many stages of gene expression allow various points of control

        this allows for very fine control, more options....

Gene expression includes all steps

        from gene to final production of phenotypic trait/function

        Fig. 17.3   Transcripition and Translational

    Condensation Effects

    Transcription

        DNA used as template for complementary RNA, not copied

            DNA is not converted into RNA

    Processing

        typical in eukaryotes, but also found in prokaryotes and some organelles

        this is an area of great current research...

    Export from nucleus

    RNA interference

        once in cytosol may be degraded, other options may exist here...

        this is another area of much research...

    Translation controls

        even if the mRNA persists, its translation can be regulated

And then there are controls at the post-translation level on the proteins!!!

        also other types of controls exist we have not raised....

Objectives:

How did Beadle and Tatum use mutants to study the biochemical pathway for arginine in *Neurospora crassa*? In what way could they determine the relative order of each mutation in the pathway? What conclusion did they reach, and in what ways it has been modified in the light of what we know today?

What is the central dogma? Based on what we covered for DNA replication in the previous lecture, what is a modification that should be made to the central dogma?

Be able to describe the process of transcription. What does it make, and what does it need in order to occur? What is the sequence of events that occur during transcription? (Items shown in figures 17.7 and 17.8 should be considered here.)

What are the three major types of RNAs made in cells? What are two minor types of RNAs? Be able to describe the functions of these different types of RNA.

In eukaryotic cells processing of the RNA is essential. What are ways in which RNA is often altered? What are the differences between a primary transcript and a mature messenger RNA? Be able to identify and describe the function(s) of the regions of mature mRNA shown in fig. 17.9. What possible role(s) might RNA processing serve? What are snRNPs and where would you expect to find them in a eukaryotic cell, and what functions do they carry out?

What is RNA interference, what enzymes are often involved, and how does this process differ from RNA processing? Be able to describe several other levels of control of gene expression that are typically seen in eukaryotes.

For review, see self-quiz questions #1 and 4 of chapter 17, and self-quiz question #8 of chapter 18.

Needed overheads and items:

Fig. 17.2, Beadle and Tatum's expt
Fig. 17.3. transcription/translation overview
Fig. 18.7, histone modifications
Fig. 17.7, stages of transcription
Fig. 17.8, initiation of transcription
Fig. 19.10, (Campbell and Reece, 2002) DNA Binding Domains
Fig. 17.7, Transcription stages
Fig. 17.7b, Transcription elongation stage
Fig. 6.8, (Alberts etal, 2002) transcription_elongation.jpg
Fig. 17.7, Transcription stages
Fig. 17.10, RNA splicing
Fig. 17.12, exons and domains in proteins.
Fig. 17.11, splicing, snRNPs
Fig. 18.11, alternate RNA splicing
Fig. 17.9, mature mRNA
Fig. 6.10, nucleus
Fig. 18.13, microRNA and RNA interference
Fig. 17.3   Transcripition and Translational

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002-Molecular Biology of the Cell. 4th edition. Figure. 6.8. Garland Science Publishing. N.Y., N.Y.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Biology. Eighth edition. Pgs. 320-322, 325-336, 347, 356-358, 362-366. Pearson/Benjamin Cummings Press. San Francisco, CA.

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Ho T-H. D- 2004-Use of Transiently expressed RNAi in dissecting hormone signaling pathways in plants- Plant Biology Seminar. Cornell University. 100 Caldwell Hall, 11:15 am, 9/10/04.

Hwang H-W., E.A. Wentzel, J.T. Mendell- 2007-A hexanucleotide element directs microRNA nuclear import- Science 315: (1/5) 97-100

Related issues:

For more on **transcription**. **RNA polymerases**. Reports of **small RNAs** that inhibit this polymerase activity. And other topics:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walker- 2002-How cells read the genome: From DNA to protein- Chapter 6, pgs. 299-374, in <u>Molecular Biology of the Cell</u>. 4th edition. Garland Science, N.Y., N.Y.

Chapman R.D., M. Heidemann, T.K. Albert, R. Mailhammer, A. Flatley, M. Meisterest, E. Kremmer, D. Eick- 2007-Transcribing RNA polymerase II is phosphorylated at CTD residue serine-7- Science 318: (#5857, 12/14) 1780-1782

Corden J.L- 2007-Seven ups the code- Science 318: (#5857, 12/14) 1735-1736

Core L.J., J.T. Lis- 2008-Transcription regulation through promoter-proximal pausing of RNA polymerase II- Science 319: (#5871, 3/28) 1791-1792

Egloff S., D. O'Reilly, R.D. Chapman, A. Taylor, K. Tanzhaus, L. Pitts, D. Eick, S. Murphy- 2007-Serine-7 of the RNA polymerase II CTD is specifically required for snRNA gene expression- Science 318: (#5857, 12/14) 1777-1779

Kapanidis A.N., E. Margeat, S.O. Ho, E. Kortkhonjia, S. Weiss, R.H. Ebright- 2006-Initial transcription by RNA polymerase proceeds through a DNA scrunching mechanism- Science 314: (11/17) 1144-1147

Kasowski M., F. Grubert, C. Heffelfinger, M. Hariharan, A. Asabere, S.M. Waszak, L. Habegger, J. Rozowsky, M. Shi, A.E. Urban, M-Y. Hong, K.J. Karczewski, W. Huber, S.M. Weissman, M.B. Gerstein, J.O. Korbel, M. Snyder- 2010-Variation in transcription factor binding among humans- Science 328: (#5975, 4/9) 232-235

Liu X., D.A., Bushnell, D. Wang, G. Calero, R.D. Komberg- 2010-Structure of an RNA polymerase II-TFIIB complex and the transcription initiation mechanism- Science 327: (#5962, 1/8) 206-209

Nechaev S., D.C. Fargo, G. Dos Santos, L. Liu, Y. Gao, K. Adelman- 2010-Global analysis of short RNAs reveals widespread promoter-proximal stalling and arrest of PolII in *Drosophila*- Science 327: (#5963, 1/15) 335-338

Revyakin A., C. Liu, R.H. Ebright, T.R. Strick- 2006-Abortive initiation and productive initiation by RNA polymerase involving DNA scrunching- Science 314: (11/17) 1139-1143

Roberts J.W- 2006-RNA polymerase, a scrunching machine- Science 314: (11/17) 1097-1098

Samara N.L., A.B. Datta, C.E. Berndsen, X. Zhang, T. Yao, R.E. Cohen, C. Wolberger- 2010-Structural insights into the assembly and function of the SAGA deubiquitinating module- Science 328: (#5981, 5/21) 1025-1029

Wassarman K.M., R.M. Saecker- 2006-Synthesis-mediated release of a small RNA inhibitor of RNA polymerase- Science 314: (12/8) 1601-1603

DNA methylation has been showed to be used to **silence transcirptional initiation**. Here is one report.

Zemach A., I.E. McDaniel, P. Silva, D. Zilberman- 2010-Genome-wide evolutionary analysis of eukaryotic DNA methylation- Science 328: (#5980, 5/14) 916-919

Here is a report of a structure in the 5' end of the forming RNA that acts as a binding site, a **riboswitch**. Its binding influences the process of transcription of the rest of the RNA.

Sudarsan N., M.C. Hammond, K.F. Block, R. Weiz, J.E. Barrick, A. Roth, R.R. Breaker- 2006-Tandem riboswitch architectures exhibit complex gene control functions- Science 314: (10/13) 300-304

During eukaryotic transcription the **nucleosomes** are often modified as the RNA polymerase moves down the template strand and new ones form so that the nucleosomes turnover.

Deal R.B., J.G. Henikoff, S. Henikoff- 2010-Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones- Science 328: (#5982, 5/28) 1161-1164

Li B., M. Gogol, M. Carey, D. Lee, C. Seidel, J.L. Workman- 2007-Combined action of PHD and Chromo domains directs the Rpd35 HDAC to transcribed chromatin- Science 316: (5/18) 1050-1054

Here are some articles that relate to **microRNAs** and the roles they play, including in **RNA interference** of gene expression.

Brown R.H- 2009-A reinnervating microRNA- Science 326: (#5959, 12/11) 1494-1495

Buchan J.R., R. Parker- 2007-The two faces of miRNA- Science 318: (#5858, 12/21) 1877-1878

Kato H., D.B. Goto, R.A. Martienssen, T. Urano, K. Furukawa, Y. Murakami- 2005-RNA polymerase II is required for RNAi-dependent heterochromatin assembly- Science 309: (7/15) 467-469

Lanford R.E., E.S. Hildebrandt-Eriksen, A. Petri, R. Persson, M. Lindow, M.E. Munk, S. Kauppinen, H. Ørum- 2010-Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection- Science 327: (#5962, 1/8) 198-201

Wienholds E., W.P. Kloosterman, E. Miska, E. Alvarez-Saavedra, E. Berezikov, E. deBruijn, H.R. Horvitz, S. Kauppinen, R.H. A. Plasterk- 2005-MicroRNA expression in zebrafish embryonic development- Science 309: (7/8) 310-311

Many of the RNAs made by transcription do not act as mRNAs. Some are called **noncoding RNAs**. Some of these can influence transcription or other processes.

Buratowski S- 2008-Gene expression - Where to start?- Science 322: (#5909, 12/19) 1804-1805

Liu F., S. Marquardt, C. Lister, S.S. Wiezewski, C. Kean- 2010-Targeted 3' processing of antisense transcripts triggers *Arabidopsis FLC* chromatin silencing- Science 327: (#5961, 1/1) 94-97

Mattick J.S- 2005-The functional genomics of noncoding RNA- Science 309: (9/2) 1527-1528

I would think that **RNA polymerase** would do transcription in one direction from the promoter... but NO... there are reports that it can transcribe upstream, or downstream, away from the promoter. Who ordered that?!

Seila A.C., J.M. Calabrese, S.S. Levine, G.W. Yeo, P.R. Rahl, R.A. Flynn, R.A. Young, P.A. Sharp- 2008-Divergent transcription from active promoters- Science 322: (#5909, 12/19) 1849-1851

For more on **nuclear pores** and how they manage to let such large polymers pass in a selective manner to achieve **export control** from the nucleus see:

Frey S., R.P. Richter, D. Görlich- 2006-FG-rich repeats of nuclear pore proteins form a three-dimensional meshwork with hydrogel-like properties- Science 314: (11/3) 815-817

Lim R.Y.H., B. Fahrenkrog, J. Köser, K. Schwarz-Herion, J. Deng, U. Aebi- 2007-Nanomechanical basis of selective gating by the nuclear pore complex- Science 318: (#5850, 10/26) 640-643

MelčáK I., A. Hoelz, G. Blobel- 2007-Structure of Nup58/45 suggests flexible nuclear pore diameter by intermolecular sliding- Science 315: (3/23) 1729-1732

Okada C., E. Yamashita, S.J. Lee, S. Shibata, J. Katahira, A. Nakagawa, Y. Yoneda, T. Tsukihara- 2009-A high-resolution structure of the pre-microRNA nuclear export machinery- Science 326: (#5957, 11/27) 1275-1279

Terry L.J., E.B. Shows, S.R. Wente- 2007-Crossing the nuclear envelope: Hierarchical regulation of nucleocytoplasmic transport- Science 318: (#5855, 11/30) 1412-1416

Work has found that sometimes **RNA splicing** results in **trans-splicing** of parts of different mRNAs to produce an new **chimeric RNA**. There are also attempts to influence RNA splicing by use of small RNAs to induce **RNA skipping** to avoid the use of certain exons to make different proteins as a result.

Li H., J. Wang, G. Mor, J. Sklar- 2008-A neoplastic gene fusion mimics trans-splicing of RNAs in normal human cells- Science 321: (#5894, 9/5) 1357-1361

Pennisi E- 2008-Hopping to a better protein- Science 322: (#5907, 12/5) 1454-1455

Rowley J.D., T. Blumenthal- 2008-The cart before the horse- Science 321: (#5894, 9/5) 1302-1304

It turns out that the mutation that led to hemophilia in Queen Victoria's family was an altered intron that lead to a change in **alternate splicing**. So how splicing is done matters.

Rogaev E.L., A.P. Griegorenka, G. Faskhutdinova, E.L.W. Kittler, Y.K. Moliaka- 2009- Genotype analysis identifies the cause of the "Royal disease"- Science 325: (#5954, 11/6) 817

This report suggests that how **alternating splicing** is done may be influenced by the pattern of **histone methylation**.

Luco R.F., Q. Pan, K. Tominaga, B.J. Blencowe, O.M. Pereira-Smith, T. Misteli- 2010- Regulation of alternative splicing by histone modifications- Science 327: (#5968, 2/19) 996-1000

The steriotype is that **prokaryotes** do not **modify** their **RNA**, but there are reports that show some bacteria do have systems to modify their RNAs. Here is one report that uses an *in vitro* system to try to get at aspects of their system.

Chan C.M., C. Zhou, R.H. Huang,- 2009-Reconstituting bacterial RNA repair and modification *in vitro*- Science 326: (#5959, 10/9) 247

Transcription can be controlled by positive feedback loops, resulting in very distinct states of expression patterns.

To T-L., N. Makeshri- 2010-Noise can induce bimodality in positive transcriptional feedback loops without bistability- Science 327: (#5969, 2/26) 1142-1145

Here is a report that in some **bacteria transcription** and DNA **replication** can both be done at the same time, just at different points along the DNA. These two molecular systems bump into each other!

Pomerantz R.T., M. O'Donnell- 2010-Direct restart of a replication fork stalled by a head-on RNA polymerase- Science 327: (#5965, 1/29) 590-592

BIO 108   2010
Day 4, Lecture #10, Title: Translation.
Text Readings: Campbell et al. (2008), pgs. 328-331, 337-344, 346-348.

Topics to cover:

       The Genetic Code
       Translation
             tRNA
             Ribosomes
             Initiation
             Elongation
             Termination
       Some Issues with Bacterial Translation
       Making Membrane or Secretory Proteins
       Summary

Will consider mainly eukaryotic translation. With a few nods to how it occurs in bacteria...

The Genetic Code
       Fig. 17.4, triplet code
       Review processes of transcription, translation (this figure skips some steps...which?)
Genetic code, Codon and anticodon
       Codons occur in part of mRNAs, their complements are in the DNA...
       Each codon is a set of 3 RNA nucleotides, used to designate each amino acid (AA)
       Will interact with anticodon in tRNA to match codon to amino acid
       note orientations; strands must be complementary AND antiparallel...

                   DNA: 3'-ACC-5'
                  mRNA: 5'-UGG-3'   (codon)
                  tRNA: 3'-ACC-5'   (anticodon)
Stop and start codons
       Fig. 17.5, genetic code
       20 AAs and $4^3 = 64$ possible codons, note how codon of 2 nucleotides would not work
       AUG = start codon and codes for methionine (MET)
       UAA, UGA, UAG = stop codons, these have proteins detect them, not tRNAs
       note redundancies, some AAs have more than one codon (redundant)
            Compare codons for Ser, Arg, Leu vs. Trp
Note importance of reading frame! Consider if lost just one nucleotide...
note this is RNA coding for AAs, not DNA!
amazingly conserved, only a few known exceptions, a characteristic of life on Earth?

Translation
Review major items needed: using information in RNA to guide formation of polypeptide
        Fig. 17.13, overview of translation
    mature mRNA, exported from nucleus and ready for use...
    AA-tRNAs, and enzymes to make these attachments (AA-tRNA synthetases)
    stop-codon-binding proteins, called release factors
    rRNAs and all the proteins with them in ribosomes
    ATP, GTP
    lots of initiation and elongation factors (proteins and RNAs) to regulate the process...

    tRNA
tRNA structure
    Fig. 17.14, tRNA structure
    3' end will have AA attached via -COOH of AA and -OH of ribose making an ester bond
    note anticodon location, will complement sequence of codon on mRNA
    a 3D structure allows for more complex intermolecular interactions than seen if linear
    obviously, there are genes in the DNA that codes for each type of tRNA...
    Also, can use some codon-anticodons more than others
        so do not need to make all 61 possible tRNAs... but need at least one type per AA
    tRNAs are often edited and modified in many ways from their original transcript
tRNA activation
    amino acid + tRNA becomes aminoacyl-tRNA (AA-tRNA)
        need to do this correctly or else genetic code is turned to mush...
    aminoacyl-tRNA synthetase for each AA-tRNA coupling
        Fig. 17.15, tRNA activation
        must check anticodon region, and amino acid
        note, uses ATP, converts it to AMP, polypeptide production costs lots of energy

    Ribosomes
Ribosomal parts   Some assembly done in nucleolus, some in cytosol...
    Fig. 17.16, ribosomal anatomy
    Large and small subunits, made of dozens of proteins, several types of rRNA molecules
    binding sites (APE, what most feel when they study this...)
        E site = exit
        P site = peptidyl site
        A site = amino acid site
    Note mRNA is read 5' to 3', relate this to transcription...
    The ribosome must bind:
        mRNA, tRNAs, stop-anticodon proteins, and many elongation factors
    Acts as a motor protein, moves along mRNA in a manner dependent on ATP hydrolysis
        analogous to myosin motor protein moving along microfilament structure...
    Some of the RNA in ribosomes has peptidyl synthetase activity, makes peptide bonds
        an example of a ribozyme activity that is very deep in life...

Initiation
Translation initiation

    Fig. 17.17, translation initiation

    small subunit interacts with mRNA

        5' cap, poly A tail, and UTR leader region on mRNA plays a role here

        the 5'-UTR region can be bound by other RNAs or proteins to influence initiation

    Met-tRNA binds start codon (AUG), this held at P site, so first AA is methionine

    large ribosome subunit locks in

    A site is open and next AA-tRNA can bind there...

Elongation
Translation Elongation

    Fig. 17.18, translation elongation

    Go through cycle

    Peptide bond formation,

        catalyzed by rRNA, a ribozyme

        perhaps one of the most important reactions in life?

    GTP used, made with phosphate from ATP (ATP + GDP = GTP + ADP)

    Note slow speed of GTP hydrolysis

        gives time for right AA-tRNA to get to A site, they come in randomly

            but once there those that can H-bond to codon will stay

        GTPases tend to be slow... this extra time allows for fewer errors

    tRNA released from E site, can be activated for use again by its AA-tRNA synthetase

    Lots of proteins involved in sub-steps of this, but not shown here....

        for instance motor proteins that moves ribosomes relative to mRNA

            similar in action to motor proteins associated with cytoskeleton?

Termination
Translation Termination

    Fig. 17.19, translation termination

    Stop codon is bound by a release factor

    Release factor, not a tRNA, binds to stop codon. This factor is a protein

        it uses water to hydrolyze the ester bond holding the peptide to the tRNA

    peptide released, what end of protein is being cut free? (c-terminus)

    disassembly of ribosome, so can assemble around another mRNA

    note mRNA can be used again and again.... mRNA guides the formation of the protein

        Translation does NOT convert mRNA <u>to</u> protein!

Polyribosomes

    Fig. 17.20, TEM of polyribosomes

        (Note: this TEM is *in vitro*, not in a cell... do students see why?)

    serial start, elongation, and termination of translation

    move along mRNA from its 5' end to 3' end....

Some Issues with Bacterial Translation
Cotranscription and translation
    Fig. 17.24, bacterial gene expression
    (This is a TEM of system *in vitro*, but similar to what happens in bacteria *in vivo*...)
    Can have one end of mRNA being transcribed, and other end being used in translation
        doing transcription on 3' end, starting translation at 5' end...
Alternate start points
    Bacteria can use, or skip the first start codon (AUG).
        Can lead to different proteins made from same mRNA,
        Often see this with shifts in reading frame, giving entirely different proteins
    So one gene may code for the same mRNA, but still get several distinct proteins formed...
First amino acid
    In many bacteria the first AA is a formylated-methionine (fMET)
        eukaryotes do not use this modified AA, but still have MET as first AA
    so if detect fMET, then know that some bacterial-made proteins are present...

Making Membrane or Secretory Proteins
Membrane and secretory proteins
    Fig. 17.21, SRP and membrane protein synthesis
    First part of polypeptide formed may have a Signal Peptide at N-terminus end
        typically less than a dozen AAs long...
    This signal is bound by Signal Recognition Particle (SRP)
        SRP includes RNA and protein, so another critical system with RNA in it...
            Obviously there is DNA that codes for this RNA...
    Once SRP binds it halts translation temporarily
    SRP receptor occurs in ER membrane, in RER and outer nuclear envelope not smooth ER
        It is a part of a translocation complex
    Translation continues once anchored to RER after the SRP is released
    Then translation is also translocating across membrane
        Cotranslocating translation....   (Consider how this would work in bacteria?)
    Can either put protein into lumen of ER, perhaps for secretion from cell
        Or can put protein into membrane of ER, to be a membrane protein
    This makes proteins for membrane or for export
    Note, signal peptide is often cut off, so may not detect it in an isolated protein...
    It can fit proteins into the membrane in various orientations and different number of passes
        Fig. 12.48, Alberts et al. (2002), single pass membrane protein.jpg
        Fig. 12.49, Alberts et al. (2002), double pass membrane protein.jpg
    Other signal sequences in peptide can code for protein delivery to
        mitochondria
        chloroplast
        microbody, etc....
    So use signal peptides to sort polypeptides into various cellular compartments...

Summary

Recall not all RNA is mRNA

       table 17.1, (Campbell and Reece, 2005) types of RNA in eukaryotic cell

       tRNA, rRNA, introns, snRNP, SRP-RNA, miRNAs, etc. (RNA in telomerase not listed)

          other forms of RNA exist...

       so not all RNA is coding for protein sequence,

          so therefore not all DNA codes for protein

          this is very expensive in terms of ATP, so it must have important functions?

       The other RNAs regulate gene expression at many levels, form binding sites,

          some act as enzymes (i.e. ribozymes)... etc.

Gene expression, have outlined steps from DNA to protein, but have not covered all of it...

       remember there can be many post-translational modifications of proteins...

       Fig. 17.26, transcription and translation

       Review stages here in protein production.

       To make this figure more complete would have to add in:

          steps for going to membrane or extracellular spaces

          RNAi processing

          etc...

(if time, review gene expression....protein biosynthesis.wma (Baum))

Objectives:

Assuming that the genetic code will be available to you, be able to take a sequence of DNA or RNA and determine what sequence of amino acids they should code for in a protein. Do all genes code for proteins? Other than proteins, what are some other gene products and what are some of the functions these other gene products carry out?

Be able to describe the process of translation. What items are needed and what happens during each stage (i.e. initiation, elongation, termination)? What molecule actually catalyzes the formation of peptide bonds? (Items in figures 17.17, 17.18, 17.19 are good to go over here.)

What are the important structural features of tRNA? What role does tRNA play in translation? With what other molecules must it interact covalently and non-covalently? How are tRNAs activated for use? What are the important structural features of mRNA that are essential for it to carry out its functions? And what functions do rRNAs carry out? Do only tRNAs interact with codons? What are release factors?

Proteins can be created in the cytosol, in some organelles, or a protein may be a membrane protein, or targeted for secretion, or to be moved into other cellular compartments. How can this protein sorting be achieved? What is an SRP and what is it made up of? What role does it play in translation? Does the translation of all proteins involve an SRP? If you have the DNA sequence of a part of a gene that is complementary to the coding region of mRNA what would you need to know if you wish to determine if it will lead to the formation of a membrane protein versus a cytosolic protein?

For review, see self-quiz questions #2, 3, 5, 7 and 8 of chapter 17.

Needed overheads and items:

Fig. 17.4, triplet code
Fig. 17.5, genetic code
Fig. 17.13, overview of translation
Fig. 17.14, tRNA structure
Fig. 17.15, tRNA activation
Fig. 17.16, ribosomal anatomy
Fig. 17.17, translation initiation
Fig. 17.18, translation elongation
Fig. 17.19, translation termination
Fig. 17.20, TEM of polyribosomes
Fig. 17.24, bacterial gene expression
Fig. 17.21, SRP and membrane protein synthesis
Fig. 12.48, Alberts et al. (2002), single pass membrane protein.jpg
Fig. 12.49, Alberts et al. (2002), double pass membrane protein.jpg
table 17.1, (Campbell and Reece, 2005) types of RNA in eukaryotic cell
Fig. 17.26, transcription and translation

If time...
protein biosynthesis.wma (Baum)

Handout:
Handout - Lecture 10.stm
Fig. 12.48, Alberts et al. (2002), single pass membrane protein.jpg
Fig. 12.49, Alberts et al. (2002), double pass membrane protein.jpg
table 17.1, (Campbell and Reece, 2005) types of RNA in eukaryotic cell

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walker- 2002n-Intracellular compartments and protein sorting. Chapter 12, Fig. 12.48-49, in <u>Molecular Biology of the Cell</u>. 4th edition. Garland Science, N.Y., N.Y.

Baum, H- The Biochemists' Songbook. "Protein Biosynthesis." Pergonom Press.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008q-From gene to protein. Chapter 17. Pages 328-331, 337-344, 346-348. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition. Table 17.1. Benjamin Cummings Press. San Francisco, CA.

Related issues:

Here are some articles that describe some work on *in vitro* **translation** systems, both some modern uses and some of the original studies that developed this system..

Ingolia N.T., S. Ghaemmaghami, J.R.S. Newman, J.S. Weissman- 2009-Genome-wide analysis *in vivo* of translation with nucleotide resolution using ribosome profiling- Science 324: (#5924, 4/10) 218-223

Isselbacker K.J- 2009-Paul C. Zamecnik (1912-2009)- Science 326: (#5958, 12/4) 1359

Mathonnet G., M.R. Fabian, Y.V. Svitkin, A. Parsyan, L. Huck, T. Murata, S. Biffo, W.C. Merrick, E. Darzynkiewicz, R.S. Pillai, W. Filipowicz, T.F. Duchaine, N. Sonnenberg- 2007-MicroRNA inhibition of translation initiation *in vitro* by targeting the CAP-binding complex eIF4F- Science 37: (#5845, 9/21) 1764-1767

Matthaei J.H., M.W. Nirenberg- 1961-Characteristics and stabilization of DNAase-sensitive protein synthesis in *E. coli* extracts- Proceedings of the National Academy of Science (USA) 47: (#10) 1580-1588

Nirenberg M.W- 1963-Cell free protein synthesis directed by messenger RNA- Methods in Enzymology 6: 17-23

Nirenberg M.W., J.H. Matthaei- 1961-The dependence of cell-free protein synthesis in *E. coli* upon naturally occurring or synthetic polyribonucleotides- Proceedings of the National Academy of Science (USA) 47: (#10) 1588-1602

Translation can be controled by microRNA (**miRNA**) binding to the 5'-UTR region of mRNAs.

Buchan J.R., R. Parker- 2007-The two faces of miRNA- Science 318: (#5858, 12/21) 1877-1878

Vasudevan S., Y. Tong, J.A. Steitz- 2007-Switching from repression to activation: MicroRNAs can up-regulate translation- Science 318: (#5858, 12/21) 1931-1934

This article describes aspects of the production of **membrane** and **secretory proteins**.

Becker T., S. Bhushan, A. Jarasch, J-P. Armache, S. Funes, F. Jussinet, J. Bumbart, T. Mielke, O. Berninghausen, K. Schulten, E. Westhof, R. Gilmore, E.C. Mandon, R. Beckmann- 2009-Sturcture of monomeric yeast and mammalian Sec61 complexes interacting with the translating ribosome- Science 326: (#5958, 12/4) 1369-1373

The **Signal Recognition Particle** (SRP) and its role in producing membrane and secretory proteins is of great interest. Here is some work relating to this system.

Bradshaw N., S.B. Neher, D.S. Booth, P. Walter- 2009-Signal sequences activate the catalytic switch of SRP RNA- Science 323: (#5910, 1/2) 127-130

Here are other studies of translation. In some cases the **5'-cap** is not needed to start translation. The various protein activities needed to **terminate translation** are still being examined. Since viral mRNA must use our translational systems, how this process is regulated so that **viral translation** is given priority is of interest.

Flint S.J., L.W. Enquist, V.R. Racaniello, A.M. Skalka- 2004-Control of translation, chapter 11, pgs. 378-410, in Principles of Virology: Molecular biology, pathogenesis, and control of animal viruses- ASM Press. Washington D.C.

Gilbert W.V., K. Zhou, T.K. Butler, J.A. Doudna- 2007-Cap-independent translation is required for starvation-induced differentiation in yeast- Science 317: (#5842, 8/31) 1224-1227

Gross T., A. Siepmann, D. Sturm, M. Windgassen, J.J. Scarcelli, M. Seedorf, C.N. Cole, H. Krebber- 2007-The DEAD-box RNA helicase Dbp5 functions in translation termination- Science 315: (2/2) 646-649

Pfingsten J.S., D.A. Costantino, J.S. Kieft- 2006-Structural basis for ribosome recruitment and manipulation by a viral IRES RNA- Science 314; (12/1) 1450-1454

It used to be thought that **translation initiation** was dependent on protein interactions and not due at all to the shape of the mRNA. But it has been found that **stemloops** and other **RNA structures**, such as **riboswitches**, in the mRNA can influence their use in translation. In other cases proteins do regulate mRNA use in translation, as in the case where mRNA use is conditional on the binding of a specific protein, such as iron-bound proteins, to it before it can be used in translation.

Barrick J.E., R.R. Breaker- 2006-The power of riboswitches- Scientific American 296: (#1, Jan.) 50-57

Klein D.J., A.R. Ferré-D'Amaré- 2006-Structural basis of glm S ribozyme activation by glucosamine-6-phosphate- Science 313: (9/22) 1752-1756

Nackley A.G., S.A. Shabalina, I.E. Tchivileva, K. Satterfield, O. Korchynskyi, S.S. Makarov, W. Maiscner, L. Distchenko- 2006-Human catechol-o-methyl transferase holotypes modulate protein expression by altering mRNA secondary structure- Science 314: (12/22) 1930-1933

Rouault T.A- 2006-If the RNA fits, use it- Science 314: (12/22) 1886-1887

Walden W.E., A.I. Selezneva, J. Dupuy, A. Volbeda, J.C. Fontecilla-Camps, E.C. Theil, K. Volz- 2006-Structure of a dual function iron regulatory protein 1 complexed with ferritin IRE-RNA- Science 314: (12/22) 1903-1908

These articles looks at events during the **elongation** phase of **translation**.

Kampmann M., G. Blobel- 2009-Nascent proteins caught in the act- Science 326: (#5958, 12/4) 1352-1353

Liljas A- 2009-Leaps in translational elongation- Science 326: (#5953, 10/30) 677-678

Here are some studies of the control of **termination** of translation, and their use of **release factors**.

Liljas A- 2008-Getting close to termination- Science 322: (#5903, 11/7) 863-864

Weixlbaumer A., H. Jin, C. Neubauer, R.M. Voorhees, S. Petry, A.C. Kelley, V. Ramakrishnau- 2008-Insights into translational termination from the structure of RF2 bound to the ribosome- Science 322: (#5903, 11/7) 953-956

The detailed structure of **ribosomes** has been worked out in a few cases. How it varies between species and during translation is of great interest.

Gao Y-G., M. Selmer, C.M. Dunham, A. Weixlbaumer, A.C. Kelley, V. Ramakrishnan- 2009-The structure of the ribosome with elongation factor G trapped in the posttranslocational state- Science 326: (#5953, 10/30) 694-699

Schmeing T.M, R.M. Voorhees, A.C. Kelly, Y-G. Gao, F.V. Murphy IV, J.R. Weir, V. Ramakrishnan- 2009-The crystal structure of the ribosome bound to EF-Tu and aminoacyl-RNA- Science 326: (#5953, 10/30) 688-694

Selmer M., C.M. Dunham, F.V. Murphy IV, A. Weixlbaumer, S. Petry, A.C. Kelley, J.R. Weir, V. Ramakrishnan- 2006-Structure of the 70S ribosome complexed with mRNA and tRNA- Science 313: (9/29) 1935-1942

It should be noted that not all proteins are made at ribosomes. Here is an article that deals with **non-ribosomal** enzyme that makes **peptide bonds**.

Tanovic A., S.A. Samel, L-O. Essen, M.A. Marahiel- 2008-Crystal structure of the termination module of a nonribosomal peptide synthetase- Science 321: (#5889, 8/1) 659-663

How transfer RNAs (**tRNA**) are made, what they actually do, and how they are modified is also of interest.

Cochella L., R. Green- 2005-An active role for tRNA in decoding beyond codon:anticodon pairing- Science 308: (5/20) 1178-1180

Daviter T., F.V. Murphy IV, V. Ramakrishnan- 2005-A renewed focus on transfer RNA- Science 308: (5/20) 1123-1124

Takano A., T. Endo, T. Yoshihisa- 2005-tRNA actively shuttles between the nucleus and cytosol in yeast- Science 309: (7/1) 140-142

The **genetic code** has some rare twists to it. In some cases one codon has been found to designate either of two amino acids depending on the local sequence in the mRNA.

Turanov A.A., A.V. Lobanov, D.E. Fomenko, H.G. Morrison, M.L. Sogin, L.A. Klobvitcher, D.L. Hattfield, V.N. Gladyshev- 2009-Genetic code supports targeted insertion of two amino acids by one codon- Science 323: (#5911, 1/9) 259-261

BIO 108   2010
Day 4, Lecture #11, Title: Control of Gene Expression I.
Text Readings: Campbell et al. (2008), pgs. 346-347, 351-358, 362-366.

Topics to cover:

What is "Expression"?
Control Points of Expression
Some Control Options seen in Bacteria
Positive versus Negative Transcriptional Controls
Operons
        Trp **Operon**
        Lac **Operon**
Summary of Control Points of Expression

What is "Expression"?
        Getting a genetic factor to have an effect. But what is "effect"?
                making RNA? showing a phenotype? obviously many intermediate steps...
                all these steps would be aspects of expression of a gene
        Various aspects of control of effects are seen
                One example, comparing two mechanisms with differing rates...
                Fig. 18.2, control of metabolic pathway
                        change rate of transcription, or change activity of existing proteins?
                        (tie in to control of *Serratia* in lab...)
                Which is fastest? Why have two such systems of control...?
        So gene expression changes tend to be slower than regulation of existing enzymes
        Note that the basis of this is proteins acting through their binding sites
                binding alters protein shape
                either alters its activity at an active site (enzyme regulation)
                        in milliseconds to minutes
                or alters activity of a DNA binding site (gene regulation) in seconds to days
                recall that some of these binding and active sites can be RNAs (i.e. ribozymes)

Control Points of Expression
        Fig. 17.25, transcription and translation in eukaryotes
        Are interested in control of expression in eukaryotes,
                but will then contrast this with options available to prokaryotes
Review points of possible control of expression (others exist), will go into details of this later...
        Fig. 18.6, opportunities for control of gene expression

Pre-transcriptional controls
> If lose the gene, then can not express it
>> Can go other way... What if gene is in excess copies?
>> So can control gene expression by number of copies of it available..
> Can alter chromatin condensation state
>> gene inactivated, turned off, via packing nucleosomes into chromatin
>> change in acetylation state of histones, acetylation decondenses chromatin

Transcriptional controls
> How often is transcription of a gene started?
>> how rapidly does transcription occur once started?
> Are the necessary inducer, transcription factors present?

RNA processing controls
> alternate splicing of primary transcript
> modification to give 5' cap and 3' tail additions
> Both are seen in eukaryotes, but also reported in some prokaryotes
> recall that this can involve microRNAs and RNAs in spliceosomes

RNA export controls
> can control if it is moved out of nucleus,
> only in eukaryotes, since prokaryotes lack a nucleus
> What if a protein binds the mature mRNA and prevents its export?
>> microRNAs can also bind mRNA and hold it in the nucleus
> What if export depends on the presence of a protein to bind the mature mRNA?
>> control of these proteins could control export?....

RNA interference and mRNA stability controls
> microRNA actions to activate dicer and slicer enzymes to remove mRNAs
>> so in cytosol can rapidly remove a mRNA, or not...
> How long does the RNA persist once made?
>> In some animal zygotes may be months-years
>> In some somatic cells may be minutes
>> Slow loss of As from poly A tail seen, related to persistence.
>> Can alter this by protein binding with the mRNAs...

Translational controls
> Does all mRNA translate equally?
> Some may interact better with Ribosomal small subunit?
> Again, proteins binding with mRNA can alter the rate of translational initiation.
> Once started, how rapidly does translation occur? Is it completed
>> is the proper reading frame used?
> Riboswitches - shape of RNA influences start of translation
>> can form stem-loops in 5'-UTR of mRNA, like in tRNA
>> this inhibits start of translation
>> binding of a protein with stem loop releaves structure, allows translation
>> (Barrick etal. 2006; Walden etal. 2006)

Stem loops can also occur in coding region, these can alter rate of translation

Rate of translation found to influence final protein shape,

even if primary sequence in polypeptide is unaltered (Nackley etal. 2006)

Post-translational controls

Recall covalent and allosteric modifications of proteins covered previously

phosphorylation, glycosylation, etc...

Another means is via compartmentation of pathways.

Does the protein fold into its proper final shape?

chaperone proteins often help other proteins fold.

Protein degradation is another means of control

Fig. 18.12, proteasome

Addition of a tag, Ubiquitin, targets for entry into Proteasomes

A region with many proteases...

Fig. 19.12x, (Campbell and Reece, 2002) TEM of proteasomes

So in gene expression there are many points of control, allows many options for regulation

Some Control Options seen in Bacteria

Some control options are used in bacteria and are rarer in eukaryotes

Now contrast above eukaryotic set of options with typical prokaryotic pattern...

this is like the pattern of gene expresson in mitochondria and chloroplasts

Very little RNA processing, though some cases are known

no RNA export controls, as no nucleus

But can act cotranscriptional/translationally

Fig. 17.24, bacterial transcription/translation

Can do both at same time at different ends of the same mRNA...

Pre-transcriptional controls

Often see gene amplification

We will cover how bacteria duplicate genes later...

into plasmids, via gene swapping etc

Supercoiling controls, are analogous to chromosome condensation in eukaryotes

Fig. 18.9x, (Campbell and Reece, 2002) *E. coli* DNA lysed cell

chromosome is circular, note the few independent plasmids in this figure

Fig. 24.23, Supercoiled DNA (Stryer, 1981)

Fig. 24.22, Supercoiled DNA (Stryer, 1981)

This shows relaxed versus supercoiled TEM of bacterial DNA

supercoiling compacts the DNA so it fits in the cell, or organelle.

at points it can be anchored to plasma membrane

this isolates regions of DNA, each region can have its own coiling

(Similarily can see regions of coiling in eukaryotic chromosomes.)

supercoiling (+) and (-)
> Fig. 18.20, DNA supercoiling (Zubay, 1983)
> Fig. 18.23, DNA supercoiling (Zubay, 1983)
> has enzymes, DNA gyrase, to put in coiling
> one way of coiling promotes stability of double helix
> other direction of coiling encourages helix to open
> this alters chromosome structure and gene expression

Translational controls
> Alternate start positions in the mRNA. Can skip first AUG and use second AUG
> > thus one mRNA can code for more than one polypeptide!

Post-translational controls
> There can be some compartments in a bacterial cell, will cover these later.
> Can regulate protein activity and stability...

Positive versus Negative Transcriptional Controls
Now deal with some Bacterial transcriptional controls, these are also in eukaryotes...
> Positive vs. negative control
> > Negative control
> > > strict on/off switch, either direction
> > > turning off a system that is normally on: repression
> > > turning on a system that is normally off: induction
> > Positive control
> > > on vs. very on, transcriptionally (like a dimmer switch on lights?)

Operons
Review structure of Operon, an arrangement of genes and control elements
> > not all bacterial genes are in operons, just most...
> > some eukaryotic genes are in operons, but most are not...
> Fig. 18.3a, *trp* operon
> one promoter site, where which enzyme must bind? (RNA polymerase)
> > may have operator sub-region,
> > one promoter for a group of genes, simplifies control?
> several genes in series
> > This means that entire pathway can be made all at once with 1:1:1:1 proteins
> > pros and cons of this system...
> > > control of functional pathway unit all at one level here, nice
> > > good if wish less total DNA? This is a bacterial tendency....
> Relate to *Serratia* pigmentation pathway? This figure shows for TRP
> > Fig. 18.2, control of metabolic pathway
> > note rapid and slower controls here. Allosteric controls versus expression controls
> > Negative feedback is on proteins versus
> > > Repression is a type of negative control of gene expression.
> Examples: *Trp* operon and *Lac* operon, we will look at some aspects of both of these

Trp Operon

Tryptophan (TRP) is needed amino acid, typically a cell must make it

Review parts of its operon

Function, make TRP, but if have TRP do not want pathway to be working as a waste...

so want an on/off switch, negative control

Fig. 18.3b, *trp* operon, repressor

Repressor, class of proteins, has its own gene

that gene must be expressed consistantly otherwise this protein is absent?

corepressor, binds to protein, alters it shape, in this case the corepressor is TRP

Activated repressor binds operator region, which blocks transcription initiation,

This system is on until turned off

so this is an example of negative control, via this repressor system

So this is called a repressible system, a type of negative control.

Lac Operon

Fig. 18.4b, *lac* operon

Lactose, milk sugar, disaccharide made up of galactose and glucose as monomers

Genes in this operon

B-galactosidase, breaks lactose down to monomers

Permease, moves lactose enter cell, so a membrane transport protein

transacetylase, function unclear (to me....)

Note promoter, with operator region

Fig. 17.17, *lac* operon operator regions (Freeman, 2008)

several sites act as operators, O1, O2, O3...

Fig. 18.4a, *lac* operon, inducible system

Want this system off until lactose is present

need on/off switch, so a negative control system

An inducible system, a type of negative control.

repressor protein sits on operator

until bound by allolactose (modified from lactose)

then change in shape of repressor protein occurs, and it comes off operator

this gives on switch, transcription can now occur

Actually the repressor binds two operator regions (O1 and O3)

forming a loop of DNA in between

Fig. 17.19, *lac* operon represson (Freeman, 2008)

Regulatory gene, not a part of this operon, must express constantly.

so the repressor gene has its own promoter, and its own expression controls...

Positive control in *lac* operon
       RNA polymerase has a low affinity for *lac* promoter
       Fig. 18.5b, *lac* operon, positive control
       When glucose is scarse, cAMP concentrations in cell goes up.
       cAMP bound by Catabolite Activator Protein (CAP)
              CAP has a gene that codes for it, and which must be expressed
              cAMP binding activates the CAP
              CAP now binds to DNA at upstream part of promoter
              Fig. 18.5, *lac* operon, positive control
       enhances binding of RNA polymerase to promoter
              (copy following on board...)
     Rate of expression of Lac operon under various conditions. In the first four columns "+" indicates the presence of the item indicated in the culture medium (glucose, lactose, and allolactose), or its condition in the cell (cAMP). In the last column the "+" indicates expression, while "+++" indiactes a high rate of expression.

Glucose Present?	Lactose Present?	Allolactose Present?	cAMP Conc. High?	Rate of expression of *lac* operon?
+	-	-	-	-
+	**+**	**+**	-	**+**
-	**+**	**+**	**+**	**+++**

        Note: first two rows show negative control, an inducible system
            last row shows positive control

Summary of Control Points of Expression
Bacterial control of gene expression:
       Pretranscriptional, via supercoiling and change in extent of gene duplication
       Transcriptional, at operon level
              Options that are transcriptional/co-translational exist.
              RNA processing exists, but is rare
       Translational, alternate start points, and riboswitches
       Post-translational, protein regulation, modification, and degradation

       (other control systems exist, but we will not go into them,
              it is not this simple even in bacteria...)

Objectives:

Be able to describe the options shown in fig. 18.6 for control of phenotypic expression in eukaryotes. Be able to describe how chromosome condensation, RNAi, riboswitches, and the ubiquitin systems fit into the stages shown in this figure.

What options of control of expression differ in bacteria versus in eukaryotes? What role does supercoiling play in the control of bacterial gene expression? What is the benefit to a bacterium in having different levels of control of expression? What would be a disadvantage of having all genes expressed all the time and equally? Be able to describe reasons and mechanisms for controlling and altering rates of gene expression.

Be able to describe the operon, its component parts, and the consequences of having several genes' expression controlled by a single promoter. Consider advantages and disadvantages of organizing genes into operons. What are the names of the various types of proteins that can interact with DNA near or in the promoter of an operon that influence the initiation of transcription? Be sure you understand how the role of a repressor protein, and expression of its gene, relates to the operon on which it acts.

The examples of the *trp* and *lac* operons are worth understanding. How does an inducible operon differ from a repressible operon, while at the same time both are examples of negative control? What is the difference between negative and positive control? Can you describe positive control of the *lac* operon? How does CAP influence the ability of RNA polymerase to initiate transcription? What are the essential preconditions for positive control of the *lac* operon to occur?

For review see self-quiz questions #1, 3, 8 and 9 of chapter 18.

Needed overheads and items:

Fig. 18.2, control of metabolic pathway
Fig. 17.26, transcription and translation in eukaryotes
Fig. 18.6, opportunities for control of gene expression
Fig. 18.12, proteasome
Fig. 19.12x, (Campbell and Reece, 2002) TEM or proteasomes
Fig. 17.24, bacterial transcription/translation
Fig. 18.9x, (Campbell and Reece, 2002) *E. coli* DNA lysed cell
Fig. 24.23, Supercoiled DNA (Stryer, 1981)
Fig. 24.22, Supercoiled DNA (Stryer, 1981)
Fig. 18.20, DNA supercoiling (Zubay, 1983)
Fig. 18.23, DNA supercoiling (Zubay, 1983)
Fig. 18.3a, *trp* operon
Fig. 18.2, control of metabolic pathway
Fig. 18.3b, *trp* operon, repressor
Fig. 18.4b, *lac* operon
Fig. 17.17, *lac* operon operator regions (Freeman, 2008)
Fig. 18.4a, *lac* operon, inducible system
Fig. 17.19, *lac* operon represson (Freeman, 2008)
Fig. 18.5b, *lac* operon, positive control
Fig. 18.5, *lac* operon, positive control

References:

Barrick J.E., R.R. Breaker- 2006-The power of riboswitches- Scientific American 296: (#1, Jan.) 50-57

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Regulation of gene expression. Chapter 18. Pages 346-347, 351-358, 362-366. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-Biology, 6th edition, Figures 18.9x, 19.12x. Benjamin Cummings Press. San Francisco, CA.

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Nackley A.G., S.A. Shabalina, I.E. Tchivileva, K. Satterfield, O. Korchynskyi, S.S. Kakarov, W. Maiscner, L. Distchenko- 2006-Human catechol-o-methyl transferase holotypes modulate protein expression by altering mRNA secondary structure- Science 314: (12/22) 1930-1933

Stryer L- 1981-Biochemistry. Second edition. Figures 24.22, 24.23. W.H. Freeman and Company. San Francisco, CA.

Walden W.E., A.I. Selezneva, J. Dupuy, A. Volbeda, J.C. Fontecilla-Camps, E.C. Theil, K. Volz- 2006-Structure of a dual function iron regulatory protein 1 complexed with ferritin IRE-RNA- Science 314: (12/22) 1903-1908

Zubay G- 1983-Biochemistry. Figures 18.20, 18.23. Addison-Wesley Publishing Company. Reading, MA.

Related issues:

For more on **supercoiling** of circular DNA see:

Higgins N.P- 1999-DNA supercoiling and its consequences for chromosome structure and function- Chapter 11, pgs 189-202, in, R.E. Charlebois editor, <u>Organization of the Prokaryotic Genome</u>. American Society for Microbiology, Washington D.C.

St. Jean A- 1999-Local genetic context, supercoiling and gene expression- Chapter 12, pgs 203-215, in, R.L. Charlebois editor, <u>Organization of the Prokaryotic Genome</u>. American Society for Microbiology, Washington D.C.

White D- 2000-Macromolecular synthesis- Chapter 10, pgs. 239-294, in, <u>The Physiology and Biochemistry of Prokaryotes</u>. 2nd edition. Oxford University Press, N.Y.

Woldringh C.L., T. Odijk- 1999-Structure of DNA with in the bacterial cell: Physics and Physiology- Chapter 10, pgs 171-187, in, R.L. Charlebois editor, <u>Organization of the Prokaryotic Genome</u>. American Society for Microbiology, Washington D.C.

For contrast, to see how eukaryotic **chromatin packing** is done see:

Hartwell L., L. Hood, M.L. Goldberg, A.E. Reynolds, L.M. Silver, R.C. Veres- 2000-The eukaryotic chromosome: An organelle for packaging and managing DNA- Chapter 11, pgs. 390-418, in, <u>Genetics: From Genes to Genomes</u>- McGraw Hill. Boston, MA.

The lac operon actually has several operator regions, and the repressor protein's binding forms a **DNA loop**.

Elf J., G-W. Li, X.S. Xie- 2007-Probing transcription factor dynamics at the single-molecule level in a living cell- Science 316: (5/25) 1191-1194

Swigon D., B.D. Coleman, W.K. Olson- 2006-Modeling the Lac repressor-operator assembly: The influence of DNA looping on Lac repressor conformation- Proceedings of the National Academy of Science (USA) 103: (#26, 6/27) 9879-9884

This article describes one type of **post-translational control** of protein functions in bacteria.

Depuydt M., S.E. Leonard, D. Vertommen, K. Denoncin, P. Morsomme, K. Wahni, J. Messens, K.S. Carroll, J-F. Collet- 2009-A periplasmic reducing system protects single cysteine residues from oxidation- Science 326: (#5956, 11/20) 1109-1111

There are many reports of how **protein stability** can be altered.

Hwang C-S., A. Schemorry, A. Varshavsky- 2010-N-terminal acetylation of cellular proteins creates specific degradation signals- Science 327: (#5968, 2/19) 973-977

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Zhang X-W., X-J. Yan, Z-R. Zhou, F-F. Yang, Z-Y. Wu, H-B. Sun, W-X. Liang, Z-X. Song, V. Lallemand-Breitenbach, M. Jeanne, Q-Y. Zhang, H-Y. Yang, Z-H. Huang, G-B. Zhou, J-H. Tong, Y. Zhang, J-H. Wu, H-Y. Hu, H. de Thé, S-J. Chen, Z. Chen- 2010-Arsenic trioxide controls the fate of PML-RARα oncoprotein by directly binding PML- Science 328: (#5975, 4/9) 240-243

For more on **operons** and control of **gene expression in bacteria** see:

Fuhrmann J., A. Schmidt, S. Spiess, A. Lehner, K. Turgay, K. Mechtler, E. Charpentier, T. Clausen- 2009-McsB is a protein arginine kinase that phosphorylates and inhibits the heat-shock regulator CtsR- Science 324: (#5932, 6/5) 1323-1327

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Ochman H., R. Raghavan- 2009-Excavating the functional landscape of bacterial cells- Science 326: (#5957, 11/27) 1200-1201

Seidelt B., C.A. Innis, D.N. Wilson, M. Gartmann, J-P. Armache, E. Villa, L.G. Trabuco, T. Becker, T. Mielke, K. Schulten, T.A. Steitz, R. Beckmann- 2009-Structural insight into nascent polypeptide chain-mediated translational stalling- Science 326: (#5958, 12/4) 1412-1415

White D- 2000-Adaptive and developmental changes- Chapter 18, pgs. 434-533, in, The Physiology and Biochemistry of Prokaryotes. 2nd edition. Oxford University Press, N.Y., N.Y.

Changes in **gene expression** in bacteria have been shown to lead to changes in **cell fate** of the bacterial cells.

Maamar H., A. Raj, D. Dubnau- 2007-Noise in gene expression determines cell fate in *Bacillus subtilis*- Science 317: (#5837, 7/27) 526-529

Mettetal J.T., A. van Ondenaarden- 2007-Necessary noise- Science 317: (#5837, 7/27) 463-464

Süel G.M., R.P. Kulkarni, J. Dworkin, J. Garcia-Ojalvo, M.B. Elowitz- 2007-Tunability and noise dependence in differentiation dynamics- Science 315: (3/23) 1716-1719

BIO 108   2010
Day 4, Lecture 12, Title: Control of Gene Expression II.
Text Readings: Campbell et al. (2008), pgs. 300-301, 320-323, 356-366, 432-442, 939-940.

Topics to cover:

Review Typical Prokaryotic Controls
Eukaryotic Controls of Gene Expression
       Chromosome Structure
       Transcription Factors
       Proximal and Distal Control Elements
       Permanent Changes to Genome
       Epigenetics
       Somatic Changes to Genome
    If Time: Review Gene Expression again...

Review Typical Prokaryotic Controls
Note: Many of these controls found in eukaryotic nuclear genes as well,
       also found in control of gene expression in mitochondria and chloroplasts...
Review a few issues of expression control...
    Chromosome coiling state
       If coiled, then promoter is not exposed.
       The bacterial chromosome anchors to proteins in membrane.
       Distinct sections can be amplified into plasmids, to alter gene copy numbers.
       So can have distinct regions with different coiling, and different gene expression.
       So chromosome structure matters.
       Coiling helps all the chromatin fit in the cell...
    Also dependent on RNA polymerase ability to bind to promoter
       negative control systems may block it
       positive control systems may enhance it
    Use of one mRNA to code for several proteins, in an entire operon
Similar issues will be seen in eukaryotic controls.

Eukaryotic Controls of Gene Expression
    Will now point out some options typically used in eukaryotic cells
       (Many of these also occur in some prokaryotic species, but often rare there....)

Chromosome Structure
Fig. 16.21, eukaryotic chromatin structure
      note common state of chromatin in interphase cell versus mitotic cell
      Also have to fit in cell...
      typical uncondensed DNA from a single human chromosome about 6 cm long
      Euchromatin: region of high current gene expression
      Heterochromatin: region of low current gene expression, often methylated histones
      histones and nucleosomes
            DNA wrapped about histone proteins, nonspecifically
            Fig. 4.30, (Alberts etal, 2002) nucleosome.jpg
            this forms s nucleosome structure
            DNA is negatively charged, so histones are positively charged...
            note regions between nucleosomes
                  here other proteins can bind, and control gene expression
            can covalently modify the histones
                  Fig. 18.7, histone modification
                        via acetylation, and alter stability
                  methylation of histones promotes their condensation
            Fig. 8.16, (Alberts etal, 2002) 36_fig_8.16.jpg
                  note 30 nm state vs. individual nucleosome state

Transcription Factors
a substance, usually a protein,
      that must bind to induce RNA polymerase to bind and carry out transcription
      may bind DNA, or RNA polymerase, both, neither...
      Fig. 19.10, (Campbell and Reece, 2002) transcription factors
            note sit in major groove of DNA
            interacts with sides of nitrogenous bases...
            This shows how protein shape relates to its ability to interact with DNA
Fig. 6.18, (Alberts etal, 2002) transcriptionfactor.jpg
      This shows how DNA can have shape altered by protein binding
      This can alter ability of other proteins to bind to DNA,
            and so lead to gene regulation
Fig. 7.27, (Alberts etal, 2002) protein_DNA_interaction.jpg
      use of weak interactions
      here ARG and side of base of a G form H-bonds
      sides of bases are available for interations with proteins
Transcription Factors are not just in eukaryotes...
Bacterial RNA polymerase does not solely bind promoter...
      sigma factor binds promoter and then RNA polymerase binds it
      once transcription starts RNA polymerase works sigma factor diffuses away

different sigma factors bind different DNA sequences
>this gives some control over which promoters
>>are bound, and which operons expressed, many found...
>Table 7.2 (Alberts etal, 2002) Sigma factors.jpg
Recall the CAP protein in the *lac* operon? Works like a transcription factor...
So transcription factors exist in bacteria as well as in eukaryotes
>are positive control systems

Proximal and Distal Control Elements
These are regions in the DNA that are close or distant from the gene
Review Eukaryotic gene structure and locate the regulatory regions
Fig. 18.8, eukaryotic gene
>Most have one gene with its own control elements
>Gene includes everything between promotor to termination region
>And region that codes for poly A signal sequence, for where mRNA will be cut
But control of gene expression can use several extra-genic regulatory regions.
Note control elements outside of promoter
>proximal control elements, within 100s-1000s of nucleotides of the gene
>Distal control elements, even act between chromosomes
Fig. 18.9, eukaryotic, enhancer system
>enhancer DNA region, an example of a distal control element
>activator, proteins that bind
>>this alters DNA shape, so close to promoter of gene
>>other proteins, DNA-bending proteins, may play a role
>>can interact with general transcription factors
>transcription complex
>>10s to 100s of proteins needed.
>>10-30% of protein-coding genes code for proteins controling expression
>so different combinations of transcription factors and activators alter phenotype
>>Fig. 18.10, cell type and transcription controls
>this is positive control which is the typical model for eukaryotes,
>>though negative control systems also occur...
Consequence of this is to make control of eukaryotic gene expression very complex

Permanent Changes to Genome
What does the genome code for?
>Fig. 21.7, DNA region types in human genome
>suggests only 1.5% of genome is "coding", and rest is "junk" DNA
>But findings now suggest over 90% of the human genome is transcribed
>>so idea that it is mostly silent is wrong (Amaral etal. 2008).
>>most of the genome codes for various RNAs, so not protein coding
Can alter the genome, and this can influence rates of gene expression, so will cover some
>options

Gene amplification,

    If can copy and reinsert gene can get more expression

    Alternative strategy is to make more mRNAs and export

    Classical example is rRNA genes

        Fig. 21.10, rRNA genes

            note how this is like an operon

            one promoter, three functional units made... done in nucleolus

        many copies of this gene allow many rRNAs made

            this is useful since does not amplify products via translation...

    Mechanisms for amplification, in eukaryotes

        (Bacteria use plasmids as a short term system... some eukaryotes also do this...)

        Transposons (also occur in bacteria)

            Fig. 21.9, transposon

            one mechanism allows copying and reinsertion

            creates new copies of a gene, so several copies available for expression

            or insertion or removal from within a gene can alter its product...

        Uneven crossing over

            (recombination also occurs in bacteria: ie. in conjugation...)

            Fig. 21.12, uneven crossing over

            can result in extra copies of a gene on one chromosome, loss from other

    Amplification implies existence of related Gene families

            Fig. 21.13, globin gene family

            duplication results in groups of related genes

            with many copies,

                extra copies are released from functional constrains

                and so see mutations accumuate and new forms of gene arise

            duplication without promoter creates pseudogenes

    Note: If can amplify, can also reduce numbers of copies...?

Epigenetics

DNA imprinting (see Gibbs, 2003)

    Fig. 15.18, DNA imprinting

    recall that methylation of DNA can alter expression of genes

    This can be inherited across generations

        even though nucleotide sequence is unaltered

    This is called an epigenetic change, ("above the genes")

        so genetic information not just in nucleotide sequence

        also in associated modifications of genetic material, and proteins...

Somatic Changes to Genome
Changes can occur in body cells, not in germ line cells,
   this can influence gene expression
   This allows cellular differentiation without altering ability to pass on genes...
Will describe just two examples of this, others occur...
Exon deletion
   Fig. 43.13, immunoglobin DNA maturation
   exon deletion to get final antibody form, cell determination by genetic change
   this happens as immune cells mature to final state...
Nuclear loss
   as in red blood cell maturation in humans...
   as in maturation of xylem and phloem cells in plants...

If Time: Review Gene Expression again...
   Almost every type of control of expression seen in bacteria is found somewhere in some species of eukaryotes, and visa versa.
   Yet do see different steriotypical patterns of controls in prokaryotes versus eukaryotes.

   (if time review gene expression process....)

Objectives:

Be able to describe several ways that control of gene expression in prokaryotes often differs from its control in eukaryotes.

How does the structure of a chromosome influence gene expression? What is an advantage of being able to highly condense eukaryotic DNA? What is the role of histones? Be able to describe epigenetic changes that influence gene expression. Would DNA tend to be more methylated in euchromatin or in heterochromatin?

Be able to describe the role of transcription factors in bacteria versus in eukaryotic gene expression. What must transcription factors be able to do to function normally? How is a eukaryotic gene and its transcriptional control similar and how is it different from a bacterial operon and how its transcription is controlled?

How do distal control elements influence gene expression in eukaryotes? What are the roles of activators and enhancers? Be able to describe in a general way how a protein can interact with DNA in order to alter gene expression.

What are mechanisms that might result in the amplification of the gene copy number in prokaryotes and in eukaryotes? What is an advantage of such a process, and what is a cost? How might gene families be produced?

Exon deletion in the maturation of immunoglobin genes is a good example of genetic deletion as a form of control of gene expression. Why can not gene deletion be a more common form of control? What would be the consequence for offspring if it were more common? Are these changes in the immunoglobin genes inherited or not?

For review, see self-quiz questions #2 and 4 of chapter 18, and self-quiz questions #4 and 5 of chapter 21.

Needed overheads and items:

Fig. 16.21, eukaryotic chromatin structure
Fig. 4.30, (Alberts etal, 2002) nucleosome.jpg
Fig. 18.7, histone modification
Fig. 8.16, (Alberts etal, 2002) 36_fig_8.16.jpg
Fig. 19.10, (Campbell and Reece, 2005) transcription factors
Fig. 6.18, (Alberts etal, 2002) transcriptionfactor.jpg
Fig. 7.27, (Alberts etal, 2002) protein_DNA_interaction.jpg
Table 7.2 (Alberts etal, 2002) Sigma factors.jpg
Fig. 18.8, eukaryotic gene
Fig. 18.9, eukaryotic, enhancer system
Fig. 18.10, cell type and transcription controls
Fig. 21.7, DNA region types in human genome
Fig. 21.10, rRNA genes
Fig. 21.9, transposon
Fig. 21.12, uneven crossing over
Fig. 21.13, globin gene family
Fig. 15.18, DNA imprinting
Fig. 43.13, immunoglobin DNA maturation

Handout:

Handout - Lecture 12.stm
Fig. 6.18, (Alberts etal, 2002) transcriptionfactor.jpg
Fig. 7.27, (Alberts etal, 2002) protein_DNA_interaction.jpg
Fig. 4.30, (Alberts etal, 2002) nucleosome.jpg

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002-<u>Molecular Biology of the Cell</u>. 4th edition. Figures 4.30, 6.18, 7.27, 8.16, and table 7.2. Garland Science. N.Y., N.Y.

Amaral P.P., M.E. Dinger, T.R. Mercer, J.S. Mattick- 2008-The eukaryotic genome as an RNA machine- Science 319: (#5871, 3/28) 1787-1789

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-<u>Biology</u>. Eighth edition. Pgs. 300-301, 320-323, 356-366, 432-442, 939-940. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figure 19.10. Benjamin Cummings Press. San Francisco, CA.

Gibbs W.W- 2003-The unseen genome-Scientific American 289: (#6) 106-113.

Related issues:

For a good overview of some means of **control of gene expression** in eukaryotes see:

> Hartwell L., L. Hood, M.L. Goldberg, A.E. Reynolds, L.M. Silver, R.C. Veres- 2000-
> Gene regulation in eukaryotes- Chapter 16, pgs. 558-589, in, <u>Genetics: From
> Genes to Genomes</u>- McGraw Hil. Boston, MA.

Here is a report of **non-coding RNAs** (ncRNA) that act to influence both chromatin condensation and transcription in eukaryotes. Many other **small RNAs** (sRNA) have been discovered, some have the complementary sense to the coding mRNAs and so are called **anti-sense RNAs**.

> Amaral P.P., M.E. Dinger, T.R. Mercer, J.S. Mattick- 2008-The eukaryotic genome as
> an RNA machine- Science 319: (#5871, 3/28) 1787-1789
> Brodersen P., L. Sakvarelidze-Achard, M. Bruun-Rasmussen, P. Dunoyer, Y.Y.
> Yamamoto, L. Sieburth, O. Voinnet- 2008-Widespread translational inhibition by
> plant miRNAs and siRNAs- Science 320: (#5880, 5/30) 1185-1190
> He Y., B. Vogelstein, V.E. Velculescu, N. Papadopoulos, K.W. Kinzler- 2008-The
> antisense transcriptomes of human cells- Science 322: (#5909, 12/19) 1855-1857
> Preker P., J. Nielsen, S. Kammler, S. Lykke-Andersen, M.S. Christensen, C.K.
> Mapendano, M.H. Schierup, T.H. Jensen- 2008-RNA exosome depletion reveals
> transcription upstream of active human promoters- Science 322: (#5909, 12/19)
> 1851-1854
> Stewart M- 2009-Nuclear export of small RNAs- Science 326: (#5957, 11/27) 1195-1196

There are also reports that changes in **RNA splicing** can lead to accumulation of **toxic RNA**s in the nucleus.

> Cooper T.A- 2009-Neutralizing toxic RNA- Science 325: (#5938, 7/17) 272-273
> Wheeler T.M., K. Sobczak, J.D. Lueck, R.J. Osborne, X. Lin, R.T. Dirksen, C.A. Thornton-
> 2009-Reversal of RNA dominance by displacement of protein sequestered on triplet
> repeat RNA- Science 325: (#5938, 7/17) 336-339

It used to be thought that **heterochromatin** was a region that was transcriptionally silent. But there are some genes being reported to be transcribed from heterochromatin areas, leading some to rethink this steriotype.

> Hoskins R.A., J.W. Carlson, C. Kennedy, D. Aceredo, M. Evans-Holm, G. Frise, K.H.
> Wan, S. Park, M. Mendez-Lago, F. Rossi, A. Villasante, P. Dimitri, G.H.
> Karpen, S.E. Celniker- 2007-Sequence finishing and mapping of *Drosophila
> melanogaster* heterochromatin- Science 316: (6/15) 1625-1628
> Smith C.D., S-Q. Shu, C.J. Mungall, G.H. Darpen- 2007-The release 5.1 annotation of
> *Drosophila melanogaster* heterochromatin- Science 316: (6/15) 1586-1591

There are many transcriptional and pre-transcriptional controls. Changes in **DNA condensation** influences gene expression in eukaryotes. Such as changes in the nucleosomes and the histones that make them up, **methylation** of sections of DNA, and uses of shared transcripitonal elements such as **cis-regulatory elements**.

Bostick M., J. KyongKim, P.O. Estève, A. Clark, S. Pradhan, S.E. Jacobsen- 2007- UHRF1 plays a role in maintaining DNA methylation in mammalian cells- Science 317: (#5845, 9/21) 1760-1764

Brown C.D., D.S. Johnson, A. Sidow- 2007-Functional architecture and evolution of transcriptional elements that drive gene coexpression- Science 317: (#5844, 9/14) 1557-1560

Chandler V.L., H. Vaucheret- 2001-Gene activation and gene silencing- Plant Physiology 125: 145-148

Coller H.A., L. Kruglyak- 2008-It's the sequence, stupid!- Science 322: (#5900, 10/17) 380-381

Krishnakumar R., M.J. Gamble, K.M. Frizzell, J.G. Berrocal, M. Kininis, W.L. Kraus- 2008-Reciprocal binding of PARP-1 and histone H1 at promoters specifies transcriptional outcomes- Science 319: (#5864, 2/8) 819-821

Kruglyak L., D.L. Stein- 2007-An embarrassment of switches- Science 317: (#5839, 8/10) 758-759

Nagano T., J.A. Mitchell, L.A. Sanz, F.M. Pauler, A.C. Ferguson-Smith, R. Feil, P. Fraser- 2008-The *Air* noncoding RNA epigenetically silences transcription by targeting G9a to chromatin- Science 322: (#5908, 12/12) 1717-1720

Perillo B., M.N. Ombra, A. Bertoni, C. Cuozzo, S. Sacchetti, A. Sasso, L. Chiariotti, A. Malorni, C. Abbondanza, E.V. Avvedimento- 2008-DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression- Science 319: (#5860, 1/11) 202-206

Saze H., A. Shiraishi, A. Maiura, T. Kakutani- 2008-Control of genic DNA methylation by a jmjC domain-containing protein in *Arabidopsis thaliana*- Science 319: (#5862, 1/25) 462-465

Singh K.B- 1998-Transcriptional regulation in plants: The importance of combinatorial control- Plant Physiology 118: (#4) 1111-1120

Swanson-Wagner R.A., R. De Cook, Y. Jia, T. Bancroft, T. Ji, X. Zhao, D. Nettleton, P.S. Schnable- 2009-Paternal dominance of trans-eQTL influences gene expression patterns in maize hybrids- Science 326: (#5956, 11/20) 1118-1120

A whole new class of **DNA-binding proteins** has been discovered.

Boch J., H. Scholze, S. Schornack, A. Landgraf, S. Hahn, S. Kay, T. Lahaye, A. Nickstadt, U. Bonas- 2009-Breaking the code of DNA binding specificity of TAL-type III effectors- Science 326: (#5959, 12/11) 1509-1512

Moscou M.J., A.J. Bogdanove- 2009-A simple cipher governs DNA recognition by TAL effectors- Science 326: (#5959, 12/11) 1501

Voytas D.F., J.K. Joung- 2009-DNA binding made easy- Science 326: (#5959, 12/11) 1491-1492

There have also been reports that the **chromosomal position** in the nucleus can influence its **condensation** state and rate of **gene expression**.

Lieberman-Aiden E., N.L. van Berkum, L. Williams, M. Imakaev, T. Ragoczy, A Telling, I. Amit, B.R. Lajoie, P.J. Sabo, M.O. Dorschner, R. Sandstrom, B. Bernstein, M.A. Bender, M. Groundine, A. Gnirke, J. Stamatoyannopoulos, L.A. Mirny, E.S. Lander, J. Dekker- 2009-Comprehensive mapping of long-range interactions reveals folding principles of the human genome- Science 326: (#5950, 10/9) 289-293

Wenner M- 2009-Nuclear architecture- Scientific American 301: (#4, Oct.) 20-22

Splicing of RNA is a level of control, and some **introns** have been found to be **self-splicing**. Much of this splicing occurs in a region of the nucleus called the **Cajal body**, a type of subnuclear body.

Kaiser T.E., R.V. Intine, M. Dundr- 2008-*De novo* formation of a subnuclear body- Science 322: (#5908, 12/12) 1713-1717

Toor N., K.S. Keating, S.D. Taylor, A.M. Pyle- 2008-Crystal structure of a self-spliced group II intron- Science 320: (#5872, 4/4) 77-82

Here are some articles that focus on the roles of **transcription factors** and their interactions with DNA in the control of gene expression.

Badis G., M.F. Berger, A.A. Philippakis, S. Talukder, A.R. Gehrke, S.A. Jaeger, E.T. Chan, G. Metzler, A. Vedenko, X Chen, H. Kuznetsov, C-F. Wang, D. Coburn, D.E. Newburger, Q. Moris, T.R. Hughes, M.L. Bulyk- 2009-Diversity and complexity in DNA recognition by transcription factors- Science 324: (#5935, 6/26) 1720-1723

Borneman A.R., T.A. Glanoulis, Z.D. Zhang, H. Yu, J. Rozowsky, M.R. Seringhaus, L.Y. Wang, M. Gerstein, M. Snyder- 2007-Divergence of transcription factor binding sites across related yeast species- Science 317: (#5839, 8/10) 815-819

Chen K-Y., B. Cong, R. Wing, J. Vrebalov, S.D. Tanksley- 2007-Changes in regulation of a transcription factor lead to autogamy in cultivated tomatoes- Science 318: (#5850, 10/26) 643-645

Lin R., L. Ding, C. Casola, D.R. Ripoll, C. Feschotte, H. Wang- 2007-Transposase-derived transcription factors regulate light signaling in *Arabidopsis*- Science 318: (#5854, 11/23) 1302-1305

Melichar H.J., K. Narayan, S.D. Der, Y. Hiraoka, N. Gardiol, G. Jeannett, W. Held, C.A. Chambers, J. Kang- 2007-Regulation of $\gamma\delta$ versus $\alpha\beta$ T lymphocyte differentiation by the transcription factor SOX13- Science 315: (1/12) 230-233

Park C.Y., R. Dolmetsch- 2006-The double life of a transcription factor takes it outside the nucleus- Science 314: (10/6) 64-65

Ranish J.A., S. Hahn- 1991-The yeast general transcription factor TFIIA is composed of two polypeptide subunits- Journal of Biological Chemistry 266: (#29) 19320-19327

Rohs R., S.M. West, A. Sosinsy, P. Liu, R.S. Mann, B. Honig- 2009-The role of DNA shape in protein-DNA recognition- Nature 461: (10/29) 1248-1253

Rowlands T., P. Baumann, s.P. Jackson- 1994-The TATA-binding protein: A general transcription factor in eukaryotes and archaebacteria- Science 264: (#5163) 1326-1329

Serizawa H., J.W. Conaway, R.C. Conaway- 1994-An oligomeric form of the large subunit of transcription factor (TF) IIE activates phosphorylation of the RNA polymerase II carboxyl-terminal domain by TFIIH- Journal of Biological Chemistry 269: (#32) 20750-20756

BIO 108   2010
Day 5, Lecture 13, Title: Viruses.
Text Readings: Campbell et al. (2008), chapter 19, and fig. 6.2.

Topics to cover:

Viral Structures
Essential Viral Functions and some types
        Lytic Cycle
        Bacterial Defenses
        Lysogenic Cycle
        Enveloped Virus (RNA virus)
        Retrovirus
Viral Mutation Rates
Uses of Virus-like items

Viral Structures
Viruses in 1800s were identified as non-filterable disease causing factors
        filtering could catch bacteria, but viruses too small
        we can filter them out today in some cases...
Viral size
        Fig. 18.2, (Campbell and Reece, 2005) virus vs. bacterium vs. eukaryotic cell sizes
            note relative sizes
        Viruses first found as items that could not be filtered out, example TMV
            a "non-filterable" infectious agent
            Fig. 19.2, TMV infected leaf
        Fig. 6.2, size ranges
        Recall that plasma membrane is about 10 nm wide, that is 0.01 µm
        Bacteria are often 0.5-3 µm across, eukaryotic cells are often 10-100s of µm across
        have to use EM to see viruses, as < 200 nanometers (0.2 µm) in size!
            TMV_electromicrograph.jpg, from my EM set
Viral types/composition
        Fig. 19.3, measles virus
        Fig. 19.3, bacteriophage
        Fig. 19.3, hepatitis virus
        Fig. 19.3, examples of viral types
        shapes can be linear, circular, polyhedrons, and bacteriophages, note size scales
        protein capsid, layer surrounding core of virus where nucleic acids and other items are
                                   present

Nucleic acids, of various types that infect animals
> Table 19.1, animal viral classes (do not memorize!, just note diversity)
> double stranded vs. single stranded types, DNA vs. RNA types
>> note sense of strand van also vary, act as template or complement strand
> can be one to several copies of virus genome per viral particle
> may or may not include certain viral enzymes

Envelope of host membrane or not, this is a biological membrane

Essential Viral Functions and some types

What a virus has to do in order to make more viruses:
> Fig. 19.4, viral reproduction cycle
> 1) attach to host cell
>> needs surface proteins that can recognize host, via specific binding sites
>> this is often done by proteins in capsid, or proteins in envelop membrane
>> For bird flu to go from targeting bird epithelia to targeting human epithelia just
>>> a two amino acid change in surface proteins of the virus is needed.
> 2) inject genetic material (nucleic acids) into host cell
>> or fuse envelope of virus with plasma membrane of host to enter cytosol
> 3) Express virus genetic information in order to...
>> take over host cell machinery.
>> create more viral protein and nucleic acids.
>>> these are processes of gene expression and replication
> 4) Assemble virus particles in host cell (i.e reproduction)
> 5) Leave host cell
>> May involve lysis of the host cell. Or exit with an envelope.

One infected cell may end up releasing hundreds to millions of virons...

Lytic Cycle

This is one pattern of a viral cycle, here it rapidly cycles through and releases viruses
> Fig. 19.5, lytic cycle
> Review steps, using a bacteriophage as an example,
>> "phage" is a virus that attacks bacteria, i.e. bacteriophage
>> need a culture of bacteria if want to grow up phage
>> Note, they will use phage DNA in their lab exercises
>>> 48502 bp long, bacteriophage lambda (λ)
>>> a good source of DNA as a size standard
>> if run out of bacteria, the virus goes dormant and can persist in dust for decades

Bacterial Defenses

How can a bacterial cell protect itself from a bacteriophage?
> Change surface items? Secrete slime/capsule to obscure self? Avoid viral receptors?
>> these may prevent viral binding to surface of bacteria, and so prevent entry

Cleave viral DNA once it is injected?
     These are DNAases which cut DNA, RNAases to cut up viral RNA
          these are called restriction enzymes, and nucleases
          Fig. 20.3, recombinant DNA production
          cut at specific sites
          bacteria protects self by altering these sites in themselves (i.e. methylation)
          Lambda (viral) DNA cut with HindIII enzyme (a restriction enzyme)
               third type from bacterium: H̲aemophilus in̲fluenzae strain d̲
               this enzyme cuts cuts at
                        5'A-AGCT T3'
                        3'T TCGA-A5'
               this cuts up original 48502 bp length of viral DNA into 8 pieces

     We use these restriction enzymes as tools for cleaving DNA at specific sites.
     Other nucleases occur that also cut DNA at specific sites: i.e. transposases...

Lysogenic Cycle
This is another type of viral cycle, they are also called "temperate" viruses
     Fig. 19.6, lysogenic cycle
     prophage, linear section of viral DNA in the host's DNA,
          later it leaves and circularizes (via ligase) and may express its genes and go lytic
     Advantages to virus to integrate its DNA into host DNA
          when host bacterium replicates it also replicates virus, new cells get the virus
          bacteria rarely cut up their own DNA, so virus DNA is protected
     This requires use of a restriction enzyme? Virus may insert at specific sites in host...
     When host stressed, virus prophage pops out and goes lytic
          There are systems the virus uses to regulate this,
               (recall negative control in operons? how could that work here?)
     Implication: Not all the genetic material need to be the host's own
          So there can be hidden virus DNA in a bacterium's DNA.
          What might insertion of viral DNA into host's DNA do to gene expression...?
     Will see that this is similar to transposons and plasmids...

Enveloped Virus (RNA virus)
Enveloped virus, has an outer membrane from host cell, often seen in eukaryotic viruses
     Fig. 19.7, enveloped virus
     This does NOT require host cell rupture
          so cell can just keep on making virus particles as long as it lives
          this can increase total number of viruses made
          virus particles released over longer time, and not in one burst...
     Also virus gets membrane envelope with many of host surface proteins,
          so looks like host membrane
          good for avoiding host immune system?

RNA viruses, the example shown here uses RNA as its genetic material
  RNA made using RNA as a template to guide its formation
    need RNA replicase, so virus must carry enzyme, or have gene for it
    RNA must act as mRNA that codes for replicase and other proteins
    Note: this is a twist to the central dogma? In what way?

  Retrovirus
Retroviruses use RNA as a template to guide the formation of DNA
  Do this via reverse transcriptase, (yet another twist to the central dogma?)
  Fig. 19.8, HIV, retrovirus
    note that can incorporate into host DNA and hide
    note role of protease coded for by viral gene, protease inhibitors use by us
  Fig. 19.8, HIV entering and leaving cell
  Note: The Provirus section. So not all DNA in eukaryotes need to be their own?
    In some species have hundreds of thousands of DNA sections thought to be
      derived from retroviruses or from DNA viruses in them.....

Viral Mutation Rates
Unlike normal replication, there is little use of error correction in viral replication
  also some viruses are single-stranded, so error correction is difficult...
  mutation rates of 1 error per 1000 nucleotides to 10000 nucleotides are typical
During infection a person's cells may put 10^{11} viral particles into the blood each day!
  So most mutated forms are made in a person over time... diversity is their strategy
The error rate for virus replication is therefore high
  so each infected cell is likely to make variants of viruses, so a diverse population
  One viral particle in one host cell, can product thousands to millions of new viral particles
    many are new variants
  thus viruses mutate rapidly, produce drug resistance, and immunoresistant strains
  this explains why flu virus keeps on coming back
    it mutates rapidly enough to alter so that
    our immune system can not recognise the new forms

Uses of Virus-like items
  What if you had a virus you could put into your prey to help your young eat it? Sounds like
    science fiction?
    There are reports of symbiotic virus that helps parasitic wasps do just that!
      Bézier etal. (2009)
    Fig. 39.28 Parasitic wasps
    Pennisi (2010) *Nasonia vitripennis*, parasitic wasps
      A popular article on parasitic wasps.
      Must evade host immune system.
      Some have coevolved with viruses.
      Virus may attach and disable the immune system of the prey.
      In some cases the virus has become endogenous to the genome of the wasp.
      So a heritable trait that is passed in the wasp genome.

A large fraction of human genome are endogenous retroviral elements. (Geuking etal. 2009)
        Fig. 46.16, Placenta of humans
        Some of these are expressed by placental cells,
            produce particles that help to diffuse, distract, mom's immune system
    This leads to the question, which came first:
            viruses, or viral-like tools?

Summary

Viruses then are either:
    specialized parasites that have been greatly reduced?
    escaped mobile genetic elements (i.e. plasmids, transposons)?
    the last remnants of the "RNA world" from before the first cellular life?
        (more on this later)
They must interact with our cells and our immune system to complete the viral cycle.
They do this by mutating enough to produce enough variation to avoid our defenses.
Different ones use many different parts of our cells, and must regulate our cells functions.
Thus we study them not just as disease agents, but to learn about aspects of biology...
    We use them in biotechnology to carry the DNA we want to introduce into an organism.

Objectives:

Relative to the size of a bacterial cell, like *E. coli,* or to a typical human skin cell, how large are viruses? How do viruses compare in size to mitochondria, or to membrane-spanning proteins?

Viruses come in various forms. Be able to describe some of the structural and compositional options often seen in different types of viruses. How does an RNA virus differ from a DNA virus? How do these differ from a retrovirus? What are bacteriophages? Some viruses carry unique enzymes with them or have genes to code for them. What are some of these enzymes and what function does each of these enzymes carry out?

What are the differences between the lytic and lysogenic viral cycles? What are prophages, and what role do they play in which of these cycles? What enzyme activities would be needed for a lysogenic virus to integrate its DNA into the host cell's DNA?

Viruses that infect human cells have to deal with our immune system. What are some ways in which viruses can get around the defenses of their host? What defenses do bacterial cells have against viruses that attack them?

For review, see self-quiz questions #1-4 and 7-8 of chapter 19.

Needed overheads and items:

Fig. 18.2, (Campbell and Reece, 2005) virus vs. bacterium vs. eukaryotic cell sizes
Fig. 19.2, TMV infected leaf
Fig. 6.2, size ranges
TMV_electromicrograph.jpg, from my EM set
Fig. 19.3, measles virus
Fig. 19.3, bacteriophage
Fig. 19.3, Hepatitis virus
Fig. 19.3, examples of viral types
Table 19.1, viral classes
Fig. 19.4, viral reproduction cycle
Fig. 19.5, lytic cycle
Fig. 20.3, recombinant DNA production
Fig. 19.6, lysogenic cycle
Fig. 19.7, enveloped virus
Fig. 19.8, HIV, retrovirus
Fig. 19.8, HIV entering and leaving cell
Fig. 39.28, Parasitic wasps
Fig. 46.16, Placenta of humans

References:

Bézier A., M. Annaheim, J. Herbiniere, C. Wetterwald, G. Gyapay, S. Bernard-Samain, P. Wincker, I. Roditi, M. Heller, M. Belghazi, R. Fpister-Wilhem, G. Periquet, C. Dupuy, E. Huguet, A-N. Volkoff, B. Lanzrein, J-M. Drezen- 2009-Polydna viruses of Braconid wasps derive from an ancestral nudivirus- Science 323: (#5916, 2/13) 926-930

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Viruses. Chapter 19. Pages 381-395, and figure 6.2. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-Biology, 7th edition, Figure 18.2. Benjamin Cummings Press. San Francisco, CA.

Geuking M.B., J. Weber, M. Dewannieux, E. Garelik, T. Heidmann, H. Hengartner, R.M. Zinkemagel, L. Hangartner- 2009-Recombination of retrotransposon and exogenous RNA virus results in nonretroviral cDNA integration- Science 323: (#5912, 1/16) 393-396

Pennisi E- 2010-The little wasp that could- Science 327: (#5963, 1/15) 260-262

Related issues:

For those who wish to get deeper into viruses including their emergence and **evolution** and various **viral traits** see:

Flint S.J., L.W. Enquist, V.R. Racaniello, A.M. Skalka- 2004-<u>Principles of Virology: Molecular biology, pathogenesis, and control of animal viruses</u>- 802 pgs. ASM Press. Washington D.C.

Populations of **viruses** have been observed to change and adapt over time. Viruses often **evolve** very quickly. The diversity of viruses in the wild are often monitored and seen to change. Here are some examples.

Hensley S.E., S.R. Das, A.L. Bailey, L.M. Schmidt, H.D. Hickman, A. Jayaraman, K. Viswanathan, R. Raman, R. Sasisekharan, J.R. Bennink, J.W. Yewdell- 2009-Hemagglutinin receptor binding avidity drives influenza A virus antigenic drift-Science 326: (#5953, 10/30) 734-736

López-Bueno A., J. Tamames, D. Velázquez, A. Maya, A. Quesada, A. Alcami- 2009-High diversity of the viral community from an Antarctic lake- Science 326: (#5954, 11/6) 859-861

Park A.W., J.M. Daly, N.S. Lewis, D.J Smith, J.L.N. Wood, B.T. Grenfell- 2009-Quantifying the impact of immune escape on transmission dynamics of influenza-Science 326: (#5953, 10/30) 726-728

Vardi A., B.A.S. Van Mooy, H.F. Fredricks, K.J. Popendorf, J.E. Ossolinski, L. Haramaty, K.D. Bidle- 2009-Viral glycosphingolipids induce lytic infection and cell death in marine phytoplankton- Science 326: (#5954, 11/6) 861-865

Vos M., P.J. Birkett, E. Birch, R.I. Griffiths, A. Buckling- 2009-Local adaptation of bacteriophages to their bacterial hosts in soil- Science 325: (#5942, 8/14) 833

Endogenous pro-viral elements in our gemone have also been associated with certain types of cancers.

Coffin J.M., J.P. Stone- 2009-A new virus for old diseases?- Science 326: (#5952, 10/23) 530-531

Lombardi V.C., F.W. Ruscotti, J. DasGupta, M.A. Pfost, K.S. Hagan, D.L. Peterson, S.K. Ruscetti, R.K. Bagni, C. Petrow-Sedowski, B. Gold, M. Dean, R.H. Silverman, J.A. Mikovits- 2009-Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome- Science 326: (#5952, 10/23) 585-589

Bird flu and **Swine flu (H1N1)** have been in the news as emerging viruses that cross over from other animals into humans.

> Cohen J- 2010-What's old is new: 1918 virus matches 2009 H1N1 strain- Science 327: (#5973, 3/26) 1563-1564
>
> Normile D- 2008-Flu virus research yields results but no magic bullet for pandemic- Science 319: (#5867, 2/29) 1178-1179
>
> Yang Y., J.D. Sugimoto, M.E. Halloran, N.E. Basta, D.L. Chao, L. Matrajt, G. Potter, E. Kenah, I.M. Longini jr- 2009-The transmissibility and control of pandemic influenza A (H1N1) virus- Science 326: (#5953, 10/30) 724-733

This article describes a neat example of how an infecting virus uses the structures of its host cell to help the **virus spread** to other cells.

> Doceul V., M. Hollinshead, L. van der Linden, G.L. Smith- 2010-Repulsion of superinfecting virons: A mechanism for rapid virus spread- Science 327: (#5967, 2/12) 873-876

These articles describes how **CMV virus** manages to **evade** our **T cells** by interfering with antigen display. Another describes how **dengue fever virus** evades our immune system.

> Dejnirattisai W., A. Jumnainsong, N. Onsirisakul, P. Fitton, S. Vasanawathana, W. Limpitikul, C. Puttihunt, C. Edwards, T. Duangchinda, S. Supasa, K. Chawansuntati, P. Malasit, J. Mongkolsalpaya, G. Screaton- 2010-Cross-reacting antibodies enhance dengue virus infection in humans- Science 328: (#5979, 5/7) 745-748
>
> Hansen S.G., C.J. Powers, R. Richards, A.B. Ventura, J.C. Ford, D. Siess, M.K. Axthelm, J.A. Nelson, M.A. Jarvis, L.J. Picker, K. Früh- 2010-Evasion of CD8^{+} T cells is critical for super infection by cytomegalovirus- Science 328: (#5974, 4/2) 102-106
>
> Hengel H., U.H. Koszinowski- 2010-A vaccine monkey wrench?- Science 328: (#5974, 4/2) 51-52

Here are a few recent articles relating to **HIV**:

> Brass A.L., D.M. Dykxhourn, Y. Benita, N. Yan, A. Engelman, R.J. Xavier, J. Lieberman, S.J.E. Hedge- 2008-Identification of host proteins required for HIV infection through a functional genomic screen- Science 319: (#5865, 2/15) 921-926
>
> Chen L., Y.D. Kwon, T. Zhou, X. Wu, S. O'Dell, L. Cavacini, A.J. Hessell, M. Pancera, M. Tang, L. Xu, Z-Y. Yang, M-Y. Zhang, J. Arthos, D.R. Burton, D.S. Dimitrov, G.J. Nabel, M.R. Posner, J. Sodroski, R. Wyatt, J.R. Mascola, P.D. Kwong- 2009-Sturctural basis of immune evasion at the site of CD4 attachment on HIV-1 gp120- Science 326: (#5956, 11/20) 1123-1127
>
> Cohen J- 2010-The ins and outs of HIV- Science 327: (#5970, 3/5) 1196-1197
>
> Cohen J- 2008-Microbicide fails to protect against HIV- Science 319: (#5866, 2/22) 1026-1027

Cohen J- 2008-Back-to-basics push as HIV prevention struggles- Science 319: (#5865, 2/15) 888

Cohen J- 2007-Reconstructing the origins of the AIDS epidemic from archieved HIV isolates- Science 318: (#5851, 11/2) 731

Fellay J., K.V. Shianna, D. Ge, S. Colombo, B. Ledergerber, M. Woule, K. Zhang, C. Gumbs, A. Castagna, A. Cassarizza, A. Cazzi-Legari, A. DeLuca, P. Easterbrook, P. Francioli, S. Mallal, J. Martinez-Picardo, J.M. Miro, N. Obel, J.P. Smith, J. Wyniger, P. Descombes, S.E. Antonarakis, N.L. Letvin, A.J. McMichael, B.F. Haynes, A. Telenti, D.B. Goldstein- 2007-A whole-genome association study of major determinants for host control of HIV-1- Science 317: (#5840, 8/17) 944-947

Hübner W., G.P. NcNerney, P. Chen, B.M. Dale, R.E. Gordon, F.Y.S. Chuang, X-D. Li, D.M. Asmuth, T. Huser, B.K. Chen- 2009-Quantitative 3D video microscopy of HIV transfer across T cell virological synapses- Science 323: (#5922, 3/27) 1743-1747

Keonig R- 2008-In South Africa, XDR TB and HIV prove a deadly combination- Science 319: (#5865, 2/15) 894-897

Klenerman P., A. McMichael- 2007-Finding footprints among the trees- Science 315: (3/15) 1505-1507

Liu S., E.A. Abbondanzier, J.W. Rausch, S.F.J. LeGrice, X. Zhuang- 2008-Slide into action: Dynamic shuttling of HIV reverse transcriptase on nucleic acid substrates- Science 322: (#5904, 11/14) 1092-1097

MacLennan C.A., J.J. Gilchrist, M.A. Gordon, A.F. Cunningham, M. Cobbold, M. Goodall, R.A. Kingsley, J.J.G. van Oosterhout, C.L. Msefula, W.L. Mandala, D.L. Leyton, J.L. Marshall, E.N. Gondwe, S. Bobat, C. López-Macías, R. Doffinger, I.R. Henderson, E.E. Zijistra, G. Dougan, M.T. Drayson, I.C.M. MacLennan, M.E. Molyneux- 2010-Dysregulated humoral immunity to nontyphoidal *Salmonella* in HIV-infected African adults- Science 328: (#5977, 4/23) 508-512

Moir S., A.S. Feuci- 2010-*Salmonella* susceptibility- Science 328: (#5977, 4/23) 439-440

Sarkar I., I. Hauber, J. Hauber, F. Buckholz- 2007-HIV-1 proviral DNA excision using an evolved recombinase- Science 316: (#5833, 6/29) 1912-1915

Triboulet R., B. Mari, Y-L. Lin, C. Chable-Bessia, Y. Bennasser, K. Lebrigand, B. Cardinaud, T. Maurin, P. Barbry, V. Baillat, J. Reynes, P. Corbeau, K-T. Jeang, M. Benkirane- 2007-Suppression of microRNA-silencing pathway by HIV-1 during virus replication- Science 315: (3/16) 1579-1582

Some viruses are associated with certain cancers. Such viruses are called **oncogenic viruses**.

Feng H., M. Shuda, Y. Chang, P.S. Moore- 2008-Clonal integration of a polyomavirus in human merkel cell carcinoma- Science 319: (#5866, 2/22) 1096-1100

Viscidi R.P., K.V. Shah- 2008-A skin cancer virus?- Science 319: (#5866, 2/11) 1049-1050

Wenner M- 2009-Virus in the brain- Scientific American 300: (#1, Jan.) 18-21

Zelkowitz R- 2009-HPV casts a wider shadow- Science 323: (#5914, 1/30) 580-581

Some recent outbreaks of **polio virus** have been traced back to the attenuated virus used as a vaccine against polio which reverted to the virulent form. Also one of the **small pox** vaccines uses attenuated virus and it also has been reported to revert on rare instances.

Kaiser J- 2007-A tame virus runs amok- Science 316: (6/8) 1418-1419

Roberts L- 2009-Polio: Looking for a little luck- Science 323: (#5915, 2/6) 702-705

Roberts L- 2007-Vaccine-related polio outbreak in Nigeria raises concerns- Science 317: (#5846, 9/28) 1842

Often viruses have proteins that can tell us about functions of our proteins. For instance, **herpes virus** has a **helicase** that works to unwind double stranded DNA. Studies of **non-coding RNA**s that are coded for by viral genes can tell us more about how viruses use miRNAs to take over the host cell, and may tell us about how our cells normally use miRNA systems.

Cullen B.R- 2007-Outwitted by viral RNAs- Science 317: (#5836, 7/20) 329-330

Myong S., M.M. Bruno, A.M. Pyle, T. Ha- 2007-Spring-loaded mechanism of DNA unwinding by hepatitis C virus N53 helicase- Science 317: (#5837, 7/27) 513-516

Here is an interesting article that describes how an infection of **rabies virus** was treated.

Willoughby R.E. jr- 2007-A cure for rabies?- Scientific American 296: (#4, April) 88-95

These articles describe some bacterial defenses against **bacteriophages**. Similar defenses have been found in eukaryotes.

Barrangou R., C. Fremaux, H. Deveau, M. Richards, P. Boyaval, S. Moineau, D.A. Romero, P. Horvath- 2007-CRISPR provides acquired resistance against viruses in prokaryotes- Science 315: (3/23) 1709-1712

Marx J- 2007-New bacterial defense against phage invaders identified- Science 315: (3/23) 1650-1651

While they are not viruses, **prions** are infectious sub-cellular items.

Angers R.C., H-E. Kang, D. Napier, S. Browning, T. Seward, C. Mathiason, A.
Balachandran, D. McKenzie, J. Castilla, C. Soto, J. Jewell, C. Graham, E.A. Hoover,
G.C. Telling- 2010-Prion strain mutation determined by prion protein conformational
compatibility and primary structure- Science 328: (#5982, 5/28) 1154-1158
Collinge J., A.R. Clarke- 2007-A general model of prion strains and their pathogenicity-
Science 318: (#5852, 11/9) 930-936
Collinge J- 2010-Prion strain mutation and selection- Science 328: (#5982, 5/28) 1111-
1112

Here is a description of the structures seen in an **RNA virus**.

Ge P., J. Tsao, S. Schein, T.J. Green, M. Luo, Z.H. Zhou- 2010-Cryo-EM model of the
bullet-shaped vesicular stromatitis virus- Science 327: (#5966, 2/5) 689-693

The **DNA** that a viral **capsid** surrounds is so highly condensed and coiled that it pushed out with up
to 50 atmospheres of pressure! Sort of a viral bomb?

Gelbart W.M., C.M. Knobler- 2009-Pressurized viruses- Science 323: (#5922, 3/27)
1682-1683

BIO 108   2010
Day 5, Lecture 14, Title: Prokaryotic Diversity.
Text Readings: Campbell et al. (2008), pgs 551-553 and Chapter 27.

Topics to cover:

>**Archaea**
>**Bacterial**
>>**Structures**
>>**Metabolic Diversity**
>>**Ecological and Life Strategies**
>>**Genetic Issues**
>>>**Conjugation**
>**Summary**

Prokaryotes account for at least a half of all the biomass on earth. Out number eukaryotes. Live in more extreme environments (down to kilometers under ground!). And do most of the metabolic processes on the planet both in terms of diversity of pathways and in amount of mass turned over.

Archaea
Two major types of prokaryotes
>Fig. 26.21, three domains
>Eubacteria, Archea are prokaryotic domains
>Eukarya is the eukaryotic domain
>Archaea are closer relatives to Eukaryotes than are Bacteria

Archaea
>These are the newest domain of life to be discovered.
>Extremophiles, extreme heat, pressure, salt conditions...
>>Fig. 27.17, heat loving prokaryotes
>Some are methanogens. This is where the methane we emit comes from...
>Membrane structure is odd, a monolayer, not a bilayer
>>Fig. 3.20, (Madigan etal, 1997) Archea_membrane.jpg
>Differences in genes, ribosomes are similar to eukaryotic type, etc..
>>unlike bacteria, have histones
>Many other differences...

Bacteria
A Domain, with many kingdoms. Oldest and most successful form of life on the planet

> **Structures**
> Nucleoid and ribosomes
>> Fig. 6.6, prokaryotic cell
>> ribosomes are smaller than eukaryotic ones, inhibited by different items
>> nucleoid is space where DNA is held, but is not membrane bound.
>
> Gram positive vs. gram negative
>> Fig. 27.3, Gram positive and gram negative cell walls
>> both take up safranin red, only gram positive retain crystal violet
>>> so gram negative are stained red
>>> so gram positive are stained violet
>>
>> both have peptidoglycan in cell wall
>> gram negative also has an extra outer membrane
>>> this gives them an extra compartment, a periplasmic region
>>
>> Fig. 27.3, gram stain example bacteria
>> A first step in classifying an unknown bacterium...
>
> Flagella
>> Fig. 27.6, bacterial flagellum
>>> how does this differ from the flagellum of a eukaryote? No cytosol in it...
>>> Recall rotary action of mitochondria F_1F_0-ATPase?
>>> This allows H^+ to pass as it twirls, so powered by proton motive force.
>
> Pili and Fimbriae
>> Fig. 27.5, fimbriae
>> Protrusions of proteins, for mobility and surface contact
>>> can put receptors at tips for specific attachments, sex pilus is one form...
>
> Endospores
>> Fig. 27.9, endospore
>> note that this allows dormancy stage
>> resistant to boiling, can lie dormant for millions of years
>
> Capsule
>> Fig. 27.4, bacterial capsule
>> Fig. 3.58, (Madigan etal, 1997) capsules.jpg
>> secreted glycosaccharides, makes a mucus layer, sticky and protective
>> can keep away viruses, or frustrate human immune system cells
>>> capsules are one type of virulence factor seen in bacteria
>
> Shapes and sizes
>> Fig. 27.00, (Campbell and Reece, 2002) Bacteria on head of a pin
>>> This pin is NOT a dirty one. Consider bacteria on surfaces.
>>
>> Fig. 27.4, Thiomargarta, (Campbell and Reece, 2002) large bacterium
>>> can become macroscopic, the arrow points to one cell!
>>
>> Fig. 27.2, prokaryotic shapes
>>> bacillus, coccus, spirillium are three common shapes, others exist...

Other internal structures

 Internal membranes, do occur in a few species, but rare

  Fig. 16.24 (Madigan etal, 1997) PSmembrane.jpg

  Fig. 27.7, prokaryotic internal membranes

  may or may not be connected to surface plasma membrane

 Gas vesicles of cyanobacteria as an example of internal organelle

  Fig. 3.64, (Madigan etal, 1997) gas_vesicle.jpg

  These give buoyancy, and help keep these cells high in water column

 Also assemblages of proteins in the cytosol.

Multicellularity

 Fig. 27.14, *Anabaena* with heterocysts

 Note this is multicellular, a filamentous form...

 Cell to cell interactions are known, even in single celled species.

  can coordinate actions via quorum sensing.

Cellular specializations

 Heterocysts of above specialize in N-fixation

 other types of cellular specializations are known in various bacteria

Metabolic Diversity

Table 27.1, nutritional modes

Run through

 autotrophs vs. heterotrophs

  types of autotrophs: chemoautotrophs, photoautotrophs

  types of heterotrophs: chemoheterotrophs, photoheterotrophs

 aerobes vs. anaerobes; obligate vs. faciltative forms

Very diverse heterotrophs

 so diverse we sometimes use bacteria in bioremediation, to cleave pollutants

Chemoautotrophs, a much more ancient form of autotrophy

Also do some important reduction reactions to "fix" various elements

 H_2SO_3 H_2S sulfur fixation

 CO_2 CH_4 carbon fixation

 N_2 NH_4 nitrogen fixation

hydrothermal vents, regions of hot reduced conditons

  hydrothermalvents.jpg (Cone, 1991)

  Fig. 25.2, hydrothermal vents

 connect reduced to oxidized conversion to

  above oxidized to reduced conversion

N-fixation, converting N_2 to NH_4 that can be used by life, and so essential for us

 Fig. 37.10, *Rhizobium* in root nodule

 Otherwise available forms of N would be very limiting

 Process (nitrogenase enzyme) is sensitive to free oxygen

  so evolved before free oxygen gas in atmosphere?

 Prokaryotes play key roles in mineral cycling in many ecosystems

Photosynthesis
>Fig. 14.70 (Alberts etal, 2002) Photosynthetic_Bacterial.jpg
>>(Note: fermentative is ancestral, did not vanish)
>Photosynthesis evolved early on
>>one lineage based on photosystems
>>>led to chloroplasts
>>>some bacteria have analogs to PSI or PSII
>>>some have both
>>other lineages based on rhodopsin proton pump
>>so several ways to do this process other than how eukaryotes do it.
>>note, light reactions first, carbon fixation systems later,
>>>oxygenic photosynthesis last
>>so several patterns of photosynthesis exist
>>>$2H_2O = O_2 + 4H^+ + 4e^{-1}$ oxidative photosynthesis
>>>$H_2S = S + 2H^+ + 2e^{-1}$ a more ancient form

Ecological and Life Strategies

Ecological issues
>With all the roles they play, they can make up entire ecosystems without eukaryotes
>A major effect on mineral cycling...
>Dispersal
>>Spores can be dispersed in air, so can be in upper atmosphere
>>Bacteria found several kilometers deep in earth

Life strategies
>Rapid life cycle, often opportunistic
>only keeps high copy number of chromosome if growing rapidly
>Under optimal conditions can divide every 15-20 min
>>So in a year can have thousands of generations
>Quantity not quality strategy, very opportunistic
>Some very complex interactions with other species.
>Distribution of metabolism
>>can share aspects of metabolism between individuals of a species
>>>or amongst individuals of distinct species
>>This implies cell-to-cell communication, and specialization...

Genetic Issues

E. coli as a genetic model organism
>roughly 5000 genes, but can have up to 8000!
>>number of genes varies with source and time
If isolate bacterium, may lose genes over time in culture
>so some essential genes, some extra for special niches?

How it handles its genes
    operons, recall operon structure
    chromosome shape
        in cell is normally supercoiled
        with subdomains open/closed
        with proteins bound to it
        copy number can vary with growth
            inefficient to replicate entire genome
        want small genome, as DNA is expensive to make
    Plasmids
        a section that can leave main chromosome
        often replicates on its own, so get multiple copies
        must have
            origin of replication, insertion/excision region
            then genes of interest
    Other ways to transfer genes exist
        Transformation: taking up DNA released from dead cells
        Transduction: movement of DNA by viruses
            Fig. 27.11, transduction
            Virus picks up bacterial genes, and moves them not viral ones
            This produces new genetic combinations
            We use this in bioengineering to insert versions of genes we want....

    Conjugation
Bacterial sex is done via Conjugation
    Fig. 27.12, bacterial conjugation
        Note the one with the fimbriae is the F+ cell...
    Fig. 27.13, bacterial conjugation
    Contact made between cells by pili, more likely under more stressful conditions
    Forms a conjugation tube (i.e. sex pilus) through which can exchange plasmids,
        or may exchange entire chromosome
        The conjugation tube is a proteinaceous tube, not membrane bound!
        this means that one individual's genetics
            can be given to another in the same generation!!
    Note F^+ and F^- types. Based on genetic factors
        F plasmid holds the needed genes in a plasmid in F+ cell
        F factor has them in the chromosome in HFR cell.
        So why do not all individuals become F^+ over time?
            Consider costs and benefits....

Summary

Summary and comparison

        Table 27.2, comparison of three domains

    IFFy statements in this table

    This table has good generalizations, but there are rare exceptions to several items

        take what is in this table as good 95% rules?

    Exceptions include:

        some bacteria have internal membranes

        have other proteins associated with their DNA, though they are not histones...

        Some bacteria have linear chromosomes

            Fig. 27.21, Lyme Disease

            Bacteria that causes this has linear chromosomes

            some streptomyces can shift from circular to linear, and back!

            My thinking is that most metabolic, molecular traits found in eukaryotes

                are also likely to be found in prokaryotes..

                That some have linear chromosomes is an example of this...

        Multicellularity and specialization seen in some bacteria

            Fig. 27.18, specialization in form

    Can form complex communities.

    Fig. 27.18, major clades of bacteria

        Read through this table to get a sense of diversity in bacteria

            (do not need to know individual groups....)

Being an entire domain, Bacteria are a very diverse group.

    Fig. 26.21, prokaryotic phylogeny

    Archea are also likely to be very diverse, but are much less well studied...

    but in biology we tend to focus on eukaryotes

        since they are often what attracts our interests. So we get a false view of life?

    Yet it is really a prokaryotic world...

Objectives:

What are some distinguishing features between the Archaea and the Bacteria and the Eukarya?

As the dominant life forms on the planet it is worth knowing the range of ecological distribution of prokarotes, the types of metabolic roles they play, and their general life strategies.

Be able to describe the structures found in bacteria, and the roles they play, such as: The nature of their cell walls, and capsules. Be able to describe the differences between gram positive and gram negative bacteria, the structural features of their chromosomes, the shapes bacterial cells often show, the function of bacterial spores and of their flagella and pili. How is the bacterial flagellum different from the eukaryotic flagellum? How can bacteria protect themselves from viruses?

Table 27.2 presents the stereotypical view of bacteria as lacking multicellularity or any sort of cellular specialization, always having circular chromosomes, lacking internal organelles, etc... You should know this stereotype, but also be able to describe exceptions to some of the features given in this table.

What are plasmids and what benefits do bacteria get from being able to form plasmids? What must a plasmid be able to do if it is going to be useful? The process of conjugation is one means that bacteria have which differs greatly from how eukaryotes carry out genetic recombination. Be able to describe this process. How can plasmids be involved in this process? Consider how the use of plasmids and conjugation can promote cellular specialization and make individuals more adaptive for conditions in which they live. Be able to describe the process of transduction and how it can alter the genetic combination in a bacterial cell. How are aspects of transduction and conjugation similar to the crossing over seen during meiosis?

For review, see self-quiz questions #1, 2, and 4 of chapter 27.

Needed overheads and items:

Fig. 26.21, prokaryotic phylogeny, three domains
Fig. 27.17, heat loving prokaryotes
Fig. 3.20, (Madigan etal, 1997) Archea_membrane.jpg
Fig. 6.6, prokaryotic cell
Fig. 27.3, Gram positive and gram negative cell walls
Fig. 27.3, gram stain example
Fig. 27.6, bacterial flagellum
Fig. 27.5, fimbriae
Fig. 27.9, endospore
Fig. 27.4, bacterial capsule
Fig. 3.58, (Madigan etal, 1997) capsules.jpg
Fig. 27.00, (Campbell and Reece, 2002) Bacteria on head of a pin
Fig. 27.4, (Campbell and Reece, 2002) Thiomargarta, large prokaryote
Fig. 27.2, prokaryotic shapes
Fig. 16.24 (Madigan etal, 1997) PSmembrane.jpg
Fig. 27.7, prokaryotic internal membranes
Fig. 3.64, (Madigan etal, 1997) gas_vesicle.jpg
Fig. 27.14, *Anabaena* with heterocysts
Table 27.1, nutritional modes
Fig. 25.2, hydrothermal vent
hydrothermalvents.jpg, (Cone, 1991)
Fig. 37.10, *Rhizobium* in root nodule
Fig. 14.70, (Alberts etal, 2002) Photosynthetic_Bacteria.jpg
Fig. 27.11, transduction
Fig. 27.12, bacterial conjugation
Fig. 27.13, bacterial conjugation
Table 27.2, comparison of three domains
Fig. 27.21, Lyme disease
Fig. 27.18, five clades of eubacteria
Fig. 26.21, prokaryotic phylogeny

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002-<u>Molecular Biology of The Cell</u>, 4th Edition. Fig. 14.70. Garland Science. N.Y., N.Y.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Bacteria and Archaea. Chapter 27, and pages 551-553. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figures 27.00, 27.4. Benjamin Cummings Press. San Francisco, CA.

Cone J- 1991-<u>Fire under the Sea</u>. 288 pgs. William Morrow Press. N.Y., N.Y.

Madigan M.T., J.M. Martinko, J. Parker- 1997-<u>Biology of Microorganisms</u>. 8th edition. Figures 3.20, 3.58, 3.64, 16.24. Prentice Hall Inc. Upper Saddle River, NJ.

Related issues:

Here is one of the first articles to report evidence of **conjugation** between bacteria, and a more recent study of conjugation.

> Babi A., A.B. Lindner, M. Vali , E.J. Stewart, M. Radman- 2008-Direct visualization of horizontal gene transfer- Science 319: (#5869, 3/14) 1533-1536
>
> Lederberg J., E.L. Tatum- 1946-Gene recombination in *Escherichia coli*- Nature 158: (#4016, 10/19) 558

We tend to think of the **bacterial genome** and **metabolism** as being "simple" but it can be rather complex. These articles find high complexity in what was thought to be a simple bacterium.

> Kühner S., V. van Noort, M.J. Betts, A. Leo-Macias, C. Bastisse, M. Rode, T. Yamada, T. Maier, S. Bader, P. Beltran-Alvarez, D. Castaño-Diez, W-H. Chen, D. Devos, M. Güell, T. Morambuena, I. Racke, V. Rybin, A. Schmidt, E. Yus, R. Aebersold, R. Herrmann, B. Böttcher, A.S. Frangakis, R.B. Russell, L. Serrana, P. Bork, A-C. Gavin- 2009-Proteome organization in a genome-reduced bacterium- Science 326: (#5957, 11/27) 1235-1240
>
> Ochman H., R. Raghavan- 2009-Excavating the functional landscape of bacterial cells- Science 326: (#5957, 11/27) 1200-1201
>
> Yus E., T. Maier, K. Michalodimitrakis, V. van Noort, T. Yamada, W-H. Chen, J.A.H. Wodke, M. Güell, S. Martínez, R. Bourgeios, S. Kühner, E. Raineri, I. Letunic, O.V. Kalinina, M. Rode, R. Herrmann, R. Gutiérrez-Gallego, R.B. Russell, A-C. Gavin, P. Bork, L. Serrano- 2009-Impact of genome reduction on bacterial metabolism and its regulation- Science 326: (#5957, 11/27) 1263-1268

There are groups of researchers who are trying to identify each and every type of bacterium that live in or on the human body. So far they have characterized the genomes of 178 types, many more to go!

> The Human Microbiome Jumpstart Reference Strains Consortium- 2010-A catalog of reference genomes from the human microbiome- Science 328: (#5981, 5/21) 994-999

Bacteria live in various environments, and influence the environment in many ways. For instance, they are reported to often be the **nucleation centers** around which rain drops and snow flakes form thus they play a major role in precipitation.

> Christner B.C., C.E. Morris, C.M. Foreman, R. Cai, D.C. Sands- 2008-Ubiquity of biological ice nucleators in snowfall- Science 319: (#5867, 2/29) 1214

Here is a nice description of a study of **segregation of DNA** and protein structures used to do this during **binary fission**.

Jensen G.J- 2009-Protein filaments caught in the act- Science 323: (#5913, 1/23) 472-473

Of great interest is what makes bacteria pathogenic to us. Identifying the **virulence factors** and **resistance to antibiotics** that promote bacterial pathogenicity in us is an ongoing area of study since knowing the mechanisms of them may allow the design of treatments to block them.

Agarwal N., W.R. Bishai- 2010-Subversion from the sidelines- Science 327: (#5964, 1/22) 417-418

Carlsson F., E.J. Brown- 2009-The art of making an exit- Science 323: (#5922, 3/27) 1678-1679

Finlay B.B- 2010-The art of bacterial warfare- Scientific American 302: (#2, Feb.) 56-63

Krantz B.A., R.A. Melnyk, S. Zhang, S.J. Juris, D.B. Lacy, Z. Wu, A. Finkelstein, R.J. Collier- 2005-A phenylalanine clamp catalyzes protein translocation through the anthrax toxin pore- Science 309: (7/29) 777-781

Liu C-I., G.Y. Liu, Y. Song, F. Yin, M.E. Hensler, W-Y. Jeng, V. Nizet, A.H-J. Wang, E. Oldfield- 2008-A cholesterol biosynthesis inhibitor blocks *Staphylococcus aureus* virulence- Science 319: (#5868, 3/7) 1391-1394

Monroe D- 2007-Deadly orbits: Why a voyage to space increased a bacterium's killing power- Scientific American 297: (#6, Dec.) 34

McNamara C., A.S. Zinkernagel, P. Macheboeuf, M.W. Cunningham, V. Nizet, P. Ghosh- 2008-Coiled-coil irregularities and instabilities in group A *Streptococcus* M1 are required for virulence- Science 319: (#5868, 3/7) 1405-1408

Stone R- 2007-Racing to defuse a bacterial time bomb- Science 317: (#5841, 8/24) 1022-1024

Volkman H.E., T.C. Pozos, J. Zhang, J.M. Davis, J.F. Rawis, L. Ramakrishnan- 2010-Tuberculosis granuloma induction via interaction of a bacterial secreted protein with host epithelium- Science 327: (#5964, 1/22) 466-469

Walsh C.T., M.A. Fischbach- 2009-New ways to squash superbugs- Scientific American 301: (#1, July) 44-51

Bacteria also work as groups. They often do this through a system called **quorum sensing**. For instance they coordinate individual cell's chances of **apoptosis** (i.e. programmed cell death) in a population. They can also do this by direct **cell-cell contact**.

Aoki S.K., R. Pamma, A.D. Hernday, J.E. Bickham, B.A. Braaten, D.A. Low- 2005- Contact-dependent inhibition of growth in *Escherichia coli*- Science 309: (8/19) 1245-1248

Kolodkin-Gal I., R. Hazan, A. Gaathon, S. Carmeli, H. Engelberg-Kulka- 2007-A linear pentapeptide is a quorum-sensing factor required for mazEF-mediated cell death in *Escherichia coli*- Science 318: (#5850, 10/26) 652-655

Kolter R- 2007-Deadly priming- Science 318: (#5850, 10/26) 578-579

Just because bacteria are small does not mean they do not have subcellular structural complexity, they do organize their proteins into distinct locations. Many protein complexes in them have been identified, such as the **carboxysomes** used for carbon fixation. or the proteins in bacterial **pili**. Other proteins are involved in time keeping, **circadian clocks**, in prokaryotic cells.

Kang H.J., F. Coulibaly, F. Ciow, T. Proft, G.N. Baker- 2007-Stabilizing isopeptide bonds revealed in gram-positive bacterial pilus structure- Science 318: (#5856, 12/7) 1625-1628

Kerfeld C.A., M.R. Sawaya, S. Tanaka, C.V. Nguyen, M. Phillips, M. Beeby, T.O. Yeates- 2005-Protein strucutres forming the shell of primitive bacterial organelles- Science 309: (8/5) 936-938

Poon A.C., J.E. Ferrell jr- 2007-A clock with a flip switch- Science 318: (#5851,11/2) 757-758

Rust M.J., J.S. Markson, W.S. Lane, D.S. Fisher, E.K. O'Shea- 2007-Ordered phosphorylation governs oscillation of a three-protein circadian clock- Science 318: (#5851, 11/2) 809-812

Shapiro L., H.H. McAdams, R. Losick- 2009-Why and how bacteria localize proteins- Science 326: (#5957, 11/27) 1225-1228

Tanaka S., C.A. Kerfeld, M.R. Sawaya, F. Cai, S. Heinhorst, G.C. Cannon, T.O. Yeates- 2008-Atomic-level models of the bacterial carboxysome shell- Science 319: (#5866, 2/22) 1083-1086

Yeates T.O., R.T. Clubb- 2007-How some pili pull- Science 318: (#5856, 12/7) 1558-1559

Bacteria have to defend themselves against bacteriophages. So they have a system of **innate immunity**, but their system involves use of changes to their DNA!

Marraffini L.A., E.J. Sontheimer- 2008-CRISPR interference limits horizontal gene transfer in *Staphylococcus* by targeting DNA- Science 322: (#5909, 12/19) 1843-1845

How much **prokaryotic diversity** is there? Some recent studies suggest we have barely begun to discover what is out there.

Bohannon J- 2007-Ocean study yields a tidal wave of microbial DNA- Science 315: (3/16) 1486-1487

Bryant D.A., A.M. Garcia-Costas, J.A. Maresca, A.G.M. Chew, C.G. Klatt, M.M. Batescon, L.J. Tallon, J. Hostetler, W.C. Nelson, J.F. Heidelberg, D.M. Ward- 2007-*Candidatus* chloracidobacterium thermophilium: An aerobic phototrophic acidobacterium- Science 317: (#5837, 7/27) 523-526

Costello E.K., C.L. Lauber, M. Hamady, N. Fierer, J.L. Gordon, R. Knight- 2009-Bacterial community variation in human body habitats across space and time- Science 326: (#5960, 12/18) 1694-1697

DeLong E.F- 2007-Life on the thermodynamic edge- Science 317: (#5836, 7/20) 327-328

Huber J.A., D.B.M. Welch, H.G. Morrison, S.M. Huse, P.R. Neal, D.A. Butterfield, M.L. Sogin- 2007-Microbial population sturctures in the deep marine biosphere- Science 318: (#5847, 10/5) 97-100

Walsh D.A., E. Zaikova, C.G. Howes, Y.C. Song, J.J. Wright, S.G. Tringe, P.D. Tortell, S.J. Hallam- 2009-Metagenome of a versatile chemolithoautotroph from expanding oceanic dead zones- Science 326: (#5952, 10/23) 578-582

Here are some articles about the Archaea. Apparently they have five distinct ways to carry out **carbon fixation** into organic matter.

Berg I.A., D. Kockelkorn, W. Buckel, G. Fuchs- 2007-A 3-hydroxypropionate/4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in archaea- Science 318: (#5857, 12/14) 1782-1786

Thauer R.K- 2007-A fifth pathway for carbon fixation- Science 318: (#5857, 12/14) 1732-1733

BIO 108   2010
Day 6, Lecture 15 , Title: Recombinant DNA I.
Text Readings: Campbell et al. (2008), chapter 20.

Topics to cover:

    Recombinant DNA for Fun and Profit...
    1) Isolating Desired DNA
        Electrophoresis
        Hybridization, Southern Blot, and FISHing
        Sequencing
    2) Making More DNA
        Polymerase Chain Reaction

Recombinant DNA for Fun and Profit...
Recombinant DNA
    A new combination of genetic information in one DNA molecule,
        new as not seen in previous ancestrial lines
    Have already seen re-combined genetics in *Sordaria* lab, transformation,
        viral transduction, and regular sexual life cycles make new combinations.
    Today this term also refers also to the introduction of
        foreign DNA from outside a species
        this can create Genetically Modified Organisms (GMO)
            Belgium Blue Bull - Sweeney 2004.jpg
Steps needed to make a GMO:
    1) Isolate and identify desired DNA.
    2) Produce more of that DNA.
    3) Create vector with the desired DNA in it.
    4) Introduce the DNA into test organisms and find which got it.
    5) Check functionality of DNA and its products...
    Will cover just first two steps today, rest next week...

1) Isolating Desired DNA
Want to get a gene of interest? Recall that there are thousands of genes in a genome.
    How hard is it to isolate DNA?
    Carlson (1998a) simple DNA isolation procedure
        DNA_isolation.jpg
    A simple method for isolation of DNA from any plant material.
    Buffer; 120 mL water (about 4 oz), 1.5 g table salt (1/4th teaspoon),
        5 g baking soda (1 tsp), 5 mL shampoo/detergent (1 tsp).
    Dice vegetable in blender with a little water, 10 second bursts or pass through
        a garlic press.
    5 mL minced vegetable plus 10 mL ice cold buffer. Stir (not shaken) about 2 min.

Either centrifuge or pass through coffee filter to produce clear solution.
Add 10 mL ice cold isopropyl alcohol (gently via plastic straw)
  to create a two phase system.
Twirl stirring rod in interface of alcohol and water to collect DNA.
Warns against using tap water.
Now have DNA, but it is all of it, too much. Need to get DESIRED DNA.
  May be one gene, or a just a small regulatory region, that is desired
Use of restriction enzymes to cut DNA in known places.
  Fig. 8.21, (Alberts etal. 2002) restriction_nucleases.jpg
  Fig. 20.3, recombinant DNA
  These enzymes are isolated from bacteria, used for defense against viruses
  Cleave selected sequences, so get fragments with known ends
But fragments are all together in one solution, so need to separate these fragments.

Electrophoresis
  DNA gel electrophoresis, many variants of this system
  Fig. 20.9, gel electrophoresis
  What is needed?
    electrical field, gel matrix, buffer, set pH and ionic levels
    sample that is soluble and charged, DNA works
  Note description of very simple systems: (Carlson, 1998b)
  Various types of electrophoresis systems exist
    paper,
    gel, of various types of matrices
      starch
      poly-acrylamide
      agarose
      agar
  Sensitivity of gel systems, two issues
    How much DNA must be present to detect?
    How small a size difference can be discerned?
      Fig. 8.23, (Alberts etal, 2002) gels.jpg
      polyacrylamide gel 1-100s of BP
      Agarose   100s-1000s- millions of BP
          up to entire chromosomes
  So now have isolated fragments of DNA.
    But which one fragment has the gene or area of interest?

Hybridization, Southern Blot, and FISHing

Hybridization

    steps in hybridization

        fig. 8.25 (Alberts etal, 2002)hybrization.jpg

        conditions used can alter interaction...

        If look for perfect match may find. nothing? why?

        why want imperfect match?

            genetic code is redundant

            AA reflected in mRNA, not DNA

                so introns do not match up

    Probe,   from AA sequence and genetic code?

        or from promoter sequence?

        Can purchase desired ssDNA from organic chemists

            properly labeled with a tag so can detect it.

        fig. 8.24c (Alberts etal, 2002) marker.jpg

    Southern Blotting

        hybridization does not work in gel

            DNA moves a bit over time if kept in gel...

        works well on paper, as can withstand process and holds DNA in one spot

            can convert dsDNA to ssDNA by heat or alkaline pH

        Fig. 20.11, Southern blotting

            transfer on to nitrocellulose paper

            (same paper used for candy wrappers!)

    FISH; Fluorescent *in situ* hybridization

        Can use probes for DNA, or for RNA

        Fig. 20.14, FISH of mRNAs in fruit fly

            can detect where the mRNA for a desired gene is expressed

            here see similar mRNAs repeat in segments of fruit fly larva

Made a guess via genetic code, or only used part of AA sequence to make probe.

Now have isolated a candidate gene for the protein

    You know what your probe hybridized with, but what about the DNA near it?

    Use this probe to find a location in the DNA (a locus)

        then can study the sequence of DNA in that area

    May want to use the probe to get to this locus and then study items in area

        such as

            promoter sequence

            regulatory sites

            sections not expressed (can students think of examples?)

Sequencing
>> need to sequence it...
> Sequencing Fragments of DNA
>> in 100s-1000s of BP at a time
>> So if DNA to be sequenced is large,
>> may need to cut it up into separate pieces and use one piece at a time...
> Sanger method
>> Fig. 20.12, Sanger method of DNA sequencing
>>> Use DNA polymerase
>>> Use DNA monomers, triphosphates
>>> Use deoxy-DNA, these stop replication where they are used.
> Done properly, makes many fragments of differing lengths
> Separate fragments via gel electrophoresis, and from it can deduce sequence

Summary: to get a candiate of a desired Gene!
> Techniques/materials needed (others exist...)
>> electrophoresis apparatus, restriction enzymes, hybridization, probes
>> Southern blotting technique, Sequencing DNA technique, etc...

2) Making More DNA

Now have one sample of a desired gene from one gel! Use it and lose it!
> Either need to isolate lots more of it, or make more of it...

> Could do lots of gel electrophoresis. Know which restriction fragment to get...

Polymerase Chain Reaction
> (PCR) uses *in vitro* DNA replication to make more of a sample
>> Fig. 20.8, PCR
>> need good specific probes to act as primers, so sequence is essential
>> note, the primers can bracket the area of DNA of interest
>> need monomer triphosphates
>> need DNA polymerase, from thermal tolerant bacteria
>> One problem, no error correction, so errors introduced
>>> error rate of DNA polymerase is about 1/10000
>>> So good only for short sections of DNA
>>> not good for large sections of chromosomes....
> This can make multiple copies (this is not the only way to make more DNA...)
> With very good probes can sometimes do PCR on original isolated DNA!!
>> And so skip many of the intermediate steps... Many variants of PCR exist....

So have an isolated region of DNA, and can make more of it.
> Have not yet made any new genetic combinations, nor have we gotten a GMO...
(Will continue next week...)

Objectives:

The techniques of Southern Blotting, electrophoresis, DNA sequencing, and the use of restriction enzymes are all essential for the finding a candidate gene from isolated DNA. Be able to describe the use of these techniques and what is needed to carry each out. What is the function of the deoxy-DNA triphosphates in the Sanger method?

What must be known to make a good probe for DNA hybridization? If you have knowledge of the amino acid sequence of a protein how could you propose a possible probe for its gene? What state must the DNA be in for it to interact with a good probe, and how do you get it into that state? Which techniques make use of some aspect of DNA hybridization?

What are some different forms of gel electrophoresis used to study DNA, and what is an advantage of each type?

Restriction enzymes are used by bacteria for what purpose? When we use these enzymes for our purposes what do they give us the ability to do? What is recombinant DNA?

Be able to describe the process of PCR and know what is needed to carry it out. What must be known before this process can be used? Be able to relate this technique to other ways we will cover in later lectures for the production of copies of a desired section of DNA.

For review, see self-quiz questions #1, 4, and 7 of chapter 20.

Needed overheads and items:

Belgium Blue Bull - Sweeney 2004.jpg
DNA_isolation.jpg (overhead relating to Carlson, 1998)
Fig. 8.21, (Alberts etal. 2002) restriction_nucleases.jpg
Fig. 20.3, recombinant DNA
Fig. 8.23, (Alberts etal, 2002) gels.jpg
Fig. 20.9, gel electrophoresis
fig. 8.25 (Alberts etal, 2002) hybrization.jpg
fig. 8.24c (Alberts etal, 2002) marker.jpg
Fig. 20.11, Southern blotting
Fig. 20.14, FISH of mRNAs in fruit fly
Fig. 20.12, Sanger method of DNA sequencing
Fig. 20.8, PCR

Handout:

Handout - Lecture 15.stm
       Alberts etal. (2002), figures 8.24c and 8.25.

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002-<u>Molecular Biology of the Cell</u>. 4th edition. Fig. 8.21, 8.23, 8.24c, 8.25. Garland Science. N.Y., N.Y.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Biotechnology. Chapter 20. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Carlson S- 1998a-Spooling the stuff of life- Scientific American 279: (#3, Sept.) 96-97

Carlson S- 1998b-Sorting molecules with electrophoresis- Scientific American 279: (#6) 110-111

Sweeney H.L- 2004-Gene doping- Scientific American 291: (#1) 62-69

Related issues:

One of the first papers to create a **genetically modified organism** (GMO). (This is the paper on which our lab exercise is based...)

Cohen S.N., A.C.Y. Chang, H.W. Boyer, R.B. Helling- 1973-Construction of biologically functional bacterial plasmids *in vitro*- Proceedings of the National Academy of Science (USA) 70: (#11) 3240-3244

Today **GMOs** are used to create food with certain nutritional qualities, desired drugs, antibodies, and other **bio-products**. This article describes some examples.

Biello D- 2010-Biofuel from bacteria- Scientific American 302: (#4, April) 21-22
Enserink M- 2008-Tough lessons from golden rice- Science 320: (#5875, 4/25) 468-471
Kaiser J- 2008-Is the drought over for pharming?- Science 320: (#5875, 4/25) 473-475

There are many different types of electrophoresis. Here is a study showing early use of electrophoresis for **DNA sequencing**, and another for its use in **DNA fingerprinting**. **Starch gel electrophoresis** is often used to separate different versions (isozymes) of the same protein as a way to look at genetic diversity of pitcher plants. Entire chromosomes can be separated, in an intact state, via **pulsed-field electrophoresis.** Also below is the citation for a more recent paper that uses a method with the cool-sounding name of **capillary electrophoresis time-of-flight mass spectrometry** (CE-TOFMS), to monitor the concentrations of metabolites in bacteria under different growth conditions.

Cole S.T., I. Saint-Girons- 1999-Bacterial genomes - all shapes and sizes- Chapter 3, pgs 35-62, in, Organization of the prokaryotic genome- R.L. Charlebois editor. American Society for Microbiology, Washington D.C.

Gill P., A.J. Jeffreys, D.J. Werrett- 1985-Forensic application of DNA 'fingerprints'- Nature 318: (Dec 12) 577-579

Godt M.J.W., J.L. Hamrick- 1996-Genetic structure of two endangered pitcher plants, *Sarracenia jonesii* and *Sarracenia oreophila* (Sarraceniaceae)- American Journal of Botany 83: (#8) 1016-1023

Ishii N., K. akahigashi, T. Baba, M. Robert, T. Saga, A. Kanai, T. Hirasawa, M. Naba, K. Hirai, A. Hoque, P.Y. Ho, Y. Kakazu, K. Sugawara, S. Igarashi, S. Harada, T. Masuda, N. Sugiyama, T. Togashi, M. Hasegawa, Y. Takai, K. Yugi, K. Arakawa, N. Twata, Y Toya, Y. Nakayama, T. Nishioka, K. Shimizu, H. Mori, M. Tomita- 2007-Multiple high-throughput analyses monitor the response of *E. coli* to perturbations- Science 316: (4/27) 593-597

Oertel W., H. Schaller- 1972-A new approach to the sequence analysis of DNA- FEBS Letters 27: (#2) 316-320

Soares C- 2010-Portrait in DNA. Can forensic analysis yield police-style sketches of suspects?- Scientific American 302: (#5, May) 14-17

The **Southern Blot** method can be used in the early search for a gene. Here is an example of this approach.

Jones N., H. Ougham, H. Thomas- 1997-Markers and mapping: We are all geneticists now- New Phytol. 137: 165-177

Here are some articles about new approaches to the **sequencing of DNA**.

Burbano H.A., E. Hodges, R.E. Green, A.W. Briggs, J. Krause, M. Meyer, J.M. Good, T. Maricic, P.L.F. Johnson, Z. Xuan, M. Rooks, A. Bhattacharjee, L. Brizuela, F.W. Albert, M. de la Rasilla, J. Fortea, A. Rosas, M. Lachmann, G.J. Hannon, S. Pääbo- 2010-Targeted investigation of the Neandertal genome by array-based sequence capture- Science 328: (#5979, 5/7) 723-725

Drmanac R., A.B. Sparks, M.J. Callow, A.L. Halpern, N.L. Burns, B.G. Kermani, P. Carnevali, I. Nazarenko, G.B. Nilsen, G. Yeung, F. Dahl, A. Fernandez, B. Staker, K.P. Pant, J. Baccash, A.P. Borcherding, A. Brownley, R. Cedeno, L. Chen, D. Chernikoff, A. Cheung, R. Chirita, B. Curson, J.C. Ebert, C.R. Hacker, R. Hartlage, B. Hauser, S. Huang, Y. Jiang, V. Karpinchyk, M. Koenig, C. Kong, T. Landers, C. Le, J. Liu, C.E. McBride, M. Morenzoni, R.E. Morey, K. Mutch, H. Perazich, K. Perry, B.A. Peters, J. Peterson, C.L. Pethiyagoda, K. Pothuraju, C. Richter, A.M. Rosenbaum, S. Roy, J. Shafto, U. Sharanhovich, K.W. Shannon, C.G. Sheppy, M. Sun, J.V. Thakuria, A. Tran, D. Vu, A.W. Zaranek, X. Wu, S. Drmanac, A.R. Oliphant, W.C. Banyai, B. Martin, D.G. Ballinger, G.M. Church, C.A. Reid- 2010-Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays- Science 327: (#5961, 1/1) 78-81

Eid J., A. Fehr, J. Gray, K. Luong, J. Lyle, G. Otto, P. Peluso, D. Rank, P. Baybayan, B. Bettman, A. Bibillo, K. Bjornson, B. Chandhuri, F. Christians, R. Cicero, S. Clark, R. Dalal, A. deWinter, J. Dixon, M. Foquet, A. Gaertner, P. Hardenbol, G. Heiner, K. Hester, D. Holden, G. Kearns, X. Kong, R. Kuse, Y. Lacroix, S. Lin, P. Lundquist, C. Ma, P. Marks, M. Maxham, D. Murphy, I. Park, T. Pham, M. Phillips, J. Roy, R. Sebra, G. Shen, J. Sorenson, A. Tomaney, K. Tarvers, M. Trulson, J. Vieceli, J. Wegener, D. Wu, A. Yang, D. Zaccarin, P. Zhao, F. Zhong, J. Korlach, S. Turner- 2009-Real-time DNA sequencing from single polymerase molecules- Science 323: (#5910, 1/2) 133-138

Pennisi E- 2010-Semiconductors inspire new sequencing technologies- Science 327: (#5970, 3/5) 1190

Here are some studies with more on **PCR**. Including one use of it to help find genes in carnivorous plants. A description of its invention. And its use in studies of gene expression in bacteria.

Cameron K.M., K.J. Wurdack, R.W. Jobson- 2002-Molecular evidence for the common origin of snap-traps among carnivorous plants- American Journal of Botany 89: (#9) 1503-1509

Carlson S- 2000-PCR at home- Scientific American 283: (#1) 102-103

Hartwell L., L. Hood, M.L. Goldberg, A.E. Reynolds, L.M. Silver, R.C. Veres- 2000- DNA at high resolution: The use of DNA cloning, PCR, and hybridization as tools of genetic analysis- Chapter 8, pgs. 262-307, in, Genetics: From Genes to Genomes- McGraw Hill. Boston, MA.

Ottesen E.A., J.W. Hong, S.R. Quake, J.R. Lendbetter- 2006-Microfluidic digital PCR enables multigene analysis of individual environmental bacteria- Science 314: (12/1) 1464-1467

Rabinow P- 1996-Making PCR. A story of Biotechnology. 190 pgs. The University of Chicago Press. Chicago, IL.

With the ability to **sequence DNA** there are now studies of specific genomic changes associated with specific genetic illnesses or ecosystem functions. For instance, one study of the **genomic changes** that typically lead to pancreatic cancer has been published. Another article describes aspectes of the new field of **ecological genomics**.

Jones S., X. Zhang, D.W. Parsons, J.C-H. Lin, R.J. Leary, P. Angenendt, P. Mankoo, H. Carter, H. Kamiyama, A. Jimeno, S-M. Hong, B. Fu, M-T. Lin, E.S. Calhoun, M. Kamiyama, K. Walter, T.N. Kolskaya, Y. Nikolsky, J. Hartigan, D.R. Smith, M. Hidalgo, S.D. Leach, A.P. Klein, E.M. Jaffee, M. Goggins, A. Maitra, C. Tacobuzio-Donahue, J.R. Eshleman, S.E. Kern, R.H. Hruban, R. Karchin, N. Papadopoulos, G. Parmigiani, B. Vogelstein, V.E. Velculescu, K.W. Kinzler- 2008- Core signaling pathways in human pancreatic cancers revealed by global genomic analyses- Science 321: (#5897, 9/26) 1801-1806

Pennisi E- 2009-Ecological genomics gets down to genes - and function- Science 326: (#5960, 12/18) 1620-1621

BIO 108   2010
Day 7, Lecture 16, Title: Fertilization.
Text Readings: Campbell et al. (2008), pages 206-207, 806-807, 812-815, 997-1003, 1021-1025, and figures 46.12 and 46.15.

Topics to cover:

> **Sperm structure and production**
> **Egg structure and production**
> **Sperm Capacitation**
> ***Zona pellucida***
> **Acrosomal reaction**
> **Plasmogamy**
> **Fast block to polyspermy**
> **Cortical reaction**
> **Completion of oogenesis and karyogamy**
> **Other issues**
> **Summary**

mutation and fertilization are two ways to get new genetic combinations
> fertilization combines factors from previous living generation, so likely to be good ones
> mutation's new factors are untested...

First will cover animal system, then look briefly at fungal and plant aspects for contrast...
Fertilization is part of sexual reproduction, recall that meiosis is also a sexual act,
> > and there is always the option to reproduce asexually,
> > but with sex get new genetic combinations much faster...

Sperm structure and production
> Two classical views of source of structure in sperm, both have a grain of truth.
> > preformation, all structures already present (we know DNA is there...)
> > epigenesis, structures form over time (we know things have to be formed...)
> Sperm parts
> > Fig. 46.12, human sperm
> > flagellum, mitochondria, centriole (this is passed to egg)
> > nucleus; holds one set of chromosomes (1N)
> > acrosome, a vesicle in the cytosol of the cell
> Sperm production, spermatogenesis
> > Fig. 46.12, spermatogenesis
> > millions made each day, quantity, not quality
> > done in testis
> > note error in this figure, early spermatids should be coenocytic...

1

Fig. 20.29, (Alberts etal. 2002) Spermatogenesis.jpg
This figure shows a more correct model
note coenocytic spermatids, 4, 8, or 16 nuclei... all in one cell initially
depends on how much early mitosis without cytokinesis before enters meiosis
most of cytosol left behind, as residual bodies, as sperm mature

Egg structure and production
Egg formation is called oogenesis
Fig. 46.12, oogenesis
interrupted meiosis, so chromosomes are condensed and do little gene expression
arrested in prophase I by 3-8 months of fetal development as primary oocyte
arrested meiosis is typical of animal kingdom, do not see this in other eukaryotes
(One exception, sea urchins do not have arrested meiosis at metaphase II...)
Fig. 20.22, (Alberts etal, 2002) oogenesis.jpg
note some cells become associated nurse cells, in the follicle in the ovary...
these send items to the developing primary oocyte
production of coat, and cortical granules in primary oocyte
cell coat is not a cell wall
but has items in it for use in species recognition
Cortical granules will play a role to block multiple sperm entry
first part of meiosis, typically in animals it creates polar body
producing quality, not quantity, as cytosol kept in one cell
(what is state of chromosomes in that polar body?)
stuck at meiosis II before fertilization
Parts of oocyte and mature egg
Fig. 20.24, (Alberts etal, 2002) oocytes.jpg
nurse/follicle cells
connected by gap junctions, so small molecules can pass
secrete factors for egg coat
also does receptor mediated endocytosis
also transfers extracellular vesicles from nurse cells to primary oocyte
maternal factors delivered to secondary oocyte; proteins, RNAs, etc...
Fig. 46.12, ovulation
note, eggs are not released by ovulation, secondary oocytes are...
so ovary does not release an egg, has not completed meiosis yet.
note presence of follicle cells (i.e "nurse" cells) coming with egg
(typical for mammals, not for many other animal species)
blastula will have to later "hatch out" of the zona and cell layer before
implanting....

Sperm Capacitation
>Changes in sperm surface as travels through uterus towards tubes
>there changes take 5-6 hours, triggered by carbonic acid in vaginal tract
>>activation of adenylyl cyclase, makes cAMP
>alters lipid and glycoproteins displayed on sperm plasma membrane
>plasma membrane's membrane potential is hyperpolarized during this time

Zona pellucida
>(most of the following is based on mouse, similar proteins with different names in other
>>animals)
>This layer is the extracellular matrix of the secondary oocyte
>>contains items that the sperm must detect so it knows it has reached this cell
>if removed from mammalian secondary oocyte,
>>then other species' sperm can enter, so is acts as a species barrier
>Fig. 20.32 (Alberts etal, 2002) cortical_reaction.jpg
>three glycoproteins in this layer include
>>ZP1, ZP2, ZP3
>receptors in sperm plasma membrane binds to carbohydrate on ZP3
>>this induces acrosomal reaction of the sperm

Acrosomal reaction
>Fig. 47.3, acrosomal cortical reactions
>Fig. 47.5, mammalian fertilization
>In sperm plasma membrane a receptor has detected signals in secondary oocyte's
>>extracellular matrix
>receptor activation leads to activation of ligand-gated sodium ion channels
>influx of sodium ions leads to depolarization of sperm plasma membrane
>membrane depolarization leads to voltage-sensitive calcium ion channel activation
>leading to calcium ion influx into sperm cytosol
>rise in cytosolic concentration of calcium ion induces exocytosis of acrosomal vesicle
>acrosomal vesicle is expelled onto *Zona pellucida*

This does several things:
>Releases hydrolytic enzymes that digests *Zona pellucida* layer
>Puts new plasma membrane on sperm surface, from vesicle, so new membrane proteins
>Then polymerization of microfilaments in sperm cytosol
>>pushes plasma membrane forward, this forms the acrosomal process
>>(how is this analogous to an amoebae's pseudopod?)
>Now new items on surface of PM of sperm in acrosomal process, or other structures,
>>are exposed and ready to be detected by receptors in plasma membrane of
>>secondary oocyte

Plasmogamy
        fusion of cytosols of two cells during fertilization
                weird thing in most animals is that sperm does NOT fuse to an egg, as no egg yet,
                        it is fusing with a secondary oocyte stuck in the middle of meiosis
        Fig. 47.3, acrosomal and cortical reaction
        Sea urchin fertilization, an exception to the above as an egg is made before fertilization.
                Note sperm-binding receptors in plasma membrane of egg
                        Bind to surface molecules on sperm acrosomal process
                        so without acrosomal reaction, hard to get plasmogamy
                When bind, these proteins signal for plasmogamy
                This process involves many proteins in a system for membrane fusion...

Fast block to polyspermy
Many sperm may encounter the same secondary oocyte, so need to avoid polyspermy
        in seconds after plasmogamy:
        Ligand-gated sodium ion channels are activated
                membrane potential changes, depolarization of it occurs
                this inhibits sperm-binding receptor activity, a fast block
        This depolarization also induces cortical reaction...
Most mammal species have lost this fast block system. But see it in sea urchins.

Cortical reaction
        Voltage sensitive calcium ion channels activated by the above depolarization
                this causes a rise in calcium ion concentration in cytosol
                this in turn induces vesicle exocytosis
                contents of vesicles alters extracellular matrix,
                        now additional sperm can not detect items in it.
                Also delivers osmotica, swelling, fertilization membrane forms in some species
        Fig. 47.4, calcium ion wave in egg
        This figure shows cortical reaction (as in sea urchin)
        calcium ions released from lumen of endoplasmic reticulum
                takes dozens of seconds
                induces fusion of cortical vesicles, exocytosis
        Fig. 20.32, (Alberts etal., 2002) corticalreaction.jpg
                Fast block, change in membrane potential
                Slow block, exocytosis and alteration of zona,
                        and/or fertilization membrane forms

Note: Similar sequence of events in sperm as in secondary oocyte
        compare to sequence of events in pre-synaptic neuron at a synapse!
Use of similar sequence of events in various situations is consistent with evolutionary model.

Completion of oogenesis and karyogamy
Recall, if not a sea urchin then there is no present egg....   not yet...
       Fig. 20.35, (Alberts etal, 2002) fertilization.jpg
       Review stages of meiosis relative to events in fertilization
           a) Secondary oocyte arrested in Metaphase II
           b) Plasmogamy done
               sperm nucleus at left,
               second polar body being formed, so meiosis II is now done
               one of these two nuclei will act as the egg nucleus
               So after plasmogamy, get egg formation !!!
           c) fusion of two nuclei, karyogamy
               arrow shows sperm tail, comes with centriole from sperm
           d) zygote diploid nucleus starts mitosis
Sperm contributes nucleus and centriole
           sperm centrioles are used as basis for future ones (non-DNA inheritance?)
           In fungi, plants, protists other organelles also often contributed
Review sequence of events
       Fig. 47.5, (Campbell and Reece, 2005) timeline of events in fertilization
       note plasmogamy and karyogamy locations in overall process of fertilization
       (Note: this figure incorrectly refers to "egg" before there is an egg!
           Unfortunately this is a common usage, so you have to double think...
           though this is correct for sea urchins...)

Other issues
Sex reversal.
           Caribbean bluehead wrasse
           Fig. 46.4, (Campbell and Reece, 2005) sex reversal
           male is large and blue colored
           if dies, then a female will become a male
           So type of gamete produced is triggered by some external signal?
Cell signaling and mating
       Recall use of signals in cell coat, receptors on sperm and secondary oocyte of animals
           see similar systems in other eukaryotes
           promotes outbreeding, and ensures species specificity...
Above is mainly animal fertilization, now consider other eukaryotes...
       Fungal sex
           Fungi have distinct mating types
           Fig. 11.2, yeast mating
               note a and α mating types, and factors, receptors for each
               this promotes cross breeding, inhibits inbreeding
           Fig. 31.20, basidiomycetes
               note this fungus has (+) and (-) mating types
               also uses chemical signals in mating
       Plant "self"-incompatibility
           Fig. 38.7, (Campbell and Reece, 2002) self incompatibility

Fig. 38.5, (Campbell and Reece, 2002) pollen
Pollen tube (male gametophyte)
     only grows to female if it is genetically distinct from
     this other plant (sporophyte) in between
     This blocks inbreeding of lines
Not really "self" fertilization as have three individuals here, and no one
     even tries to mate with itself?
     Rather this is in breeding in a lineages?
Plant double fertilization and sperm checking will be described next lecture...

Summary

Use of cell signaling systems that we have seen before.
     Recall events in pre-synaptic axon terminals of neurons. Also at immuno-synapse.
     This is another example of "unity" in life.
     If you study biochemical systems you will see this reuse of systems many times.
Fertilization
     Promotes new genetic combinations. (When is this good? When is it bad?)
     Has to ensure species-specificity... Hence signal/receptor usage...

Objectives:

What role do nurse cells play in the growth of the animal primary oocyte before fertilization? What is the state of the genetic material in the primary oocyte during this time? What effect does this have on gene expression, and how does this relate to the need for nurse cells? What are polar bodies? How many are made, and what state are the chromosomes in in each as it is formed?

Know the structures found in sperm and secondary oocyte cells that are critical to fertilization (Figures 46.12, 47.3, and 47.5 may help here).

What must happen to an animal sperm cell before it can fuse with a secondary oocyte? What roles do capacitation and the acrosomal reaction play in preparing a sperm cell for plasmogamy?

Fertilization can be divided into the substages of plasmogamy and karyogamy. Be able to describe these substages in the process of animal fertilization.

How does the secondary oocyte select sperm of its own species? How do the slow and fast blocks to polyspermy differ and how does each work? What is the role of the *zona pellucida*? Be able to describe the steps in the acrosomal and cortical reactions. How are the processes of the acrosomal and cortical reactions similar to what occurs in a presynaptic neuron? Other than its genetic material, what do many sperm of many species also contribute to the resulting zygote?

Consider the benefits and costs of the use of fertilization in a species' life cycle: Under what conditions might asexual reproduction be favored? Be able to discuss the use of cell signaling systems by animals, and compare this to systems used in fungi and plants that attempt to undergo mating.

For review, see self-quiz question #8 of chapter 46, and #1 of chapter 47.

Needed overheads and items:

Fig. 46.12, human sperm
Fig. 46.12, spermatogenesis
Fig. 20.29, (Alberts etal, 2002) spermatogenesis.jpg
Fig. 46.12, oogenesis
Fig. 20.22, (Alberts etal, 2002) oogenesis.jpg
Fig. 20.24, (Alberts etal, 2002) oocytes.jpg
Fig. 46.12, ovulation
Fig. 20.32, (Alberts etal., 2002) corticalreaction.jpg
Fig. 47.3, acrosomal and cortical reactions
Fig. 47.5, mammalian fertilization
Fig. 47.3, arcrosomal and cortical reactions
Fig. 47.4, calcium ion distribution with fertilization
Fig. 20.32, (Alberts etal., 2002) corticalreaction.jpg
Fig. 20.35, (Alberts etal, 2002) fertilization.jpg
Fig. 47.5, (Campbell and Reece, 2005) timeline of events in fertilization
Fig. 46.4, (Campbell and Reece, 2005) sex reversal
Fig. 11.2, yeast mating
Fig. 31.19, basidiomycetes
Fig. 38.7, (Campbell and Reece, 2002) self incompatibility
Fig. 38.5, (Campbell and Reece, 2002) pollen

Handout:

Handout - Lecture 18.stm
Fig. 20.22, (Alberts etal, 2002) oogenesis.jpg
Fig. 20.32, (Alberts etal., 2002) corticalreaction.jpg

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter- 2002-<u>Molecular Biology of the Cell</u>. 4th edition. Figures 20.22, 20.24, 20.29, 20.32, 20.35. Garland Science. N.Y., N.Y.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-<u>Biology</u>. Eighth edition. Pages 206-207, 806-807, 812-815, 997-1003, 1021-1025, and figures 46.12 and 46.15. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition, Figures 46.4 and 47.5. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figures 38.5, 38.7. Benjamin Cummings Press. San Francisco, CA.

Related issues:

For a review of the details of **animal fertilization** see:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walker- 2002-Germ cells and
fertilization. Chapter 20, pgs. 1127-1156, in <u>Molecular Biology of the Cell</u>.
4th edition. Garland Science, N.Y., N.Y.

Terasaki M- 1998-Imaging of Echinoderm fertilization- Molecular Biology of the Cell-
9: 1609-1612

The contributions of the oocyte to the zygote have been found to include the **nucleolus** structure.
Also the **centriole** may contain important items, some of which might be genetic factors
independent of the nucleus.

Alliegro M.C., M.A. Alliegro, P.E. Palazzo- 2006-Centrosome-associate of RNA in surf
clam oocytes- PNAS 103: (24) 9034-9038

Ogushi S., C. Palmieri, H. Fulka, M. Saitou, T. Miyano, J. Fulka jr- 2008-The maternal
nucleolus is essential for early embryonic development in mammals- Science
319: (#5863, 2/1) 613-616

How animal **sperm** find their ways through life has been studied.

Riedel I.H., K. Kruse, J. Howard- 2005-A self-organized vortex array of
hydrodynamically entrained sperm cells- Science 309: (7/8) 300-303

In vitro fertilization is used to produce millions of humans every year. Some non-reproductive
uses of this technology include the potential to create human **stem cells**.

Holden C- 2006-Scientists create human stem cell line from "dead" embryos- Science
313: (9/29) 1869

Vogel G- 2006-Scientists derive line from single embryo cell- Science 313: (8/25)
1031

This article explores issues of **male infertility** in older animals.

Dean R., M.B. Bonsall, T. Pizzari- 2007-Aging and sexual conflict- Science 316:
(4/20) 383-384

Some **parasites** alter the behavior of the animals they infect, including the sexual behavior and the
actual sex of the infected individual!

Zimmer C- 2000-A precise horror- Chapter 4, pgs. 79-117, in <u>Parasite Rex</u>. Simon &
Schuster Inc. N.Y., N.Y.

It used to be thought that the mature mammalian ovary had a limited supply of cells to produce **oocytes**. But recent reports have suggested that somatic cells may migrate to the ovaries and act as a source of new oocytes.

Vogel G- 2005-Controversial study finds an unexpected source of oocytes- Science 309: (7/29) 678-679

Unlike the relative ease of doing it in many animal species, *in vitro* **fertilization** procedures for most **plant** species are rather difficult to carry out since isolating the egg is rather tough. Here are reports of achievement of this feat.

Faure J-E., C. Dumas- 2001-Fertilization in flowering plants. New approaches for an old story- Plant Physiology 125: 102-104

Kranz E., J. Kumlehn- 1999-Angiosperm fertilization, embryo and endosperm development- Plant Science 142: 183-197

BIO 108   2010
Day 7, Lecture 17, Title: Angiosperm life cycle.
Text Readings: Campbell et al. (2008), pgs. 252-253, 600-606, 618-621, 625-634, 801-811.

Topics to cover:

> **Angiosperm Strategy**
> **Homosporous Life Cycle to Heterosporous Life Cycle**
> **Sporophyte**
> > **Flower Structures and Sporangia**
> > **Meiosis and Spore Production**
> **Ovule and Female Gametophyte Development**
> **Anther Sac and Male Gametophyte Development**
> > **Pollen and Pollination**
> **Double Fertilization makes Zygote and Endosperm**
> **Growth of Endosperm and Sporophytic Embryo**
> **Seeds and Fruits**
> **If Time: Slide Show**

Angiosperm Strategy
Gymnosperms and angiosperms are the current living seeded plants... were others in the past
> Unlike pines, angiosperms often use animals to help them achieve
> > pollination and dispersion
> so need to interact well with animals... play with their minds?
> Why should pre-meds study plants?
> > Plant's experiments in plant/animal interactions
> > > drugs, poisons, perfumes and foods to attract pollinators, repell herbivores
> > Therefore plants are a source of drugs: ethnobotany, clinical work, etc...
> > Use of plants to make other items. Genetically modified crops...

Homosporous Life Cycle to Heterosporous Life Cycle
> Review homosporous life cycle; typical of non-seeded plants...
> > Homosporous, one type of sporangium, makes spore of one size in a sporangium
> > spore (1N), does mitosis to become gametophyte
> > gametophyte, via mitosis makes archegonium + antheridium, to make gametes
> > gametes (1N), egg and sperm, do fertilization to make a zygote
> > zygote (2N), via mitosis will become a sporophyte
> > sporophyte (2N), multicellular, in sporangia do meiosis and make spores
> contrast to heterosporous gymnosperm and angiosperm (flowering plant) life cycle
> > Heterosporous, two types of sporangia, makes spores of two distinct sizes
> > microsporangium, in which meiosis makes microspores
> > megasporangium, in which meiosis makes megaspores
> > sporophyte (2N), is the generation that has sporangia and does meiosis

1

spores (1N), two types, each will do mitosis, and differ in developmental fate
male gametophyte (1N), microgametophyte, does mitosis to make sperm
female gametophyte (1N), megagametophyte, does mitosis to make egg
male and female gametophytes (microgamteophyte and megagametophyte)
in angiosperms the gametophytes are so reduced to so few cells
that can not form complete archegonia and antheridia,
but still make gametes
gametes (1N), egg and sperm meet to do fertilization to make a zygote
zygote (2N), uses mitosis to grow and develop into the sporophyte
embryo (2N), a name for a young sporophyte

Sporophyte
Now will consider how the above fits into flowering plants

Flower Structures and Sporangia
Flower is part of the sporophyte generation
Flower structures
4 whorls of modified leaves: sepals, petals, stamen, carpel
stamen: filament, anthers
carpel (also called pistil): stigma, style, ovary, ovule
complete vs. incomplete flowers: 4 whorls present vs. less than all 4
perfect vs. imperfect flowers: stamen and pistil present vs. at least one lacking
What part of the sporophyte stage of flowering plants does sex?
(If you define "sex" as fertilization then, none...
if you include meiosis as part of sexual life cycle then the sporangia do it...)
What is sexual reproduction?
(fusion of egg and sperm, but also change in ploidy to 1N?)
What do the reproductive structures of animals produce?   (gametes)
Reproductive structures in plants that make the gametes are?
(antheridia and archegonia, or items derived from them, on gametophytes)
Reproductive structures in plants that make spores are? (sporangia on sporophyte)
The text notes the sporophytic reproductive structures, but tends to ignore the
gametophytic reproductive structures... Both are needed for this sexual life cycle.

Meiosis and Spore Production
A sporophyte has sporangia in which it does meiosis to make spores
Sporangia here are: anther sacs, and a region in the ovule
Note megaspore will stay in ovule
Microspore is in anther and may be easily released...
Making spores is a sexual reproductive act.
Note these spores grow into gametophytic plants,
this is reproductive as increasing numbers of individuals
it is NOT asexual reproduction as there is change in ploidy in spore formation
Remember, no gametes yet, so no fertilization, just lots of anticipation...

Ovule and Female Gametophyte Development
(Note: Just as animals have variations on sex, there are variations on how gametophytes develop in the angiosperms. So what is presented here is the "typical" model.)
The megaspore is held in the ovule, it does mitosis to become a female gametophyte
        Again, spore production was a sexual reproductive act
            just one that animals do not do...
     Consider ovule structures, recall ovule is part of sporophyte and has a sporangium
        ovule: funiculus, connects ovule to inner wall of ovary
            integuments, wrap around ovule, will later become seed coat
            micropyle, opening in integuments, through which pollen tube later enters
            nucellus, region of ovule that acts as the megasporangium
     Four megaspore nuclei made in one cell, three degrade, one large megaspore left
        going for quality here, not quantity, all the cytosol ends up with one nucleus
Development of Female Gametophyte
        megaspore does mitosis, mitosis, mitosis = 8 nuclei produced, put in 7 cells
        mature female gametophyte has 7 cells: (typically, other versions of this exist...)
            antipodals, three cells near to funiculus
            synergids, two lateral cells near to micropyle
            egg, in between the synergids
            central cell, has two nuclei (N+N) called polar nuclei
                (recall dikaryotic state of fungi?)
        Have we made a gamete? (yes, so this is a sexual organism!)
        By mitosis or by meiosis? (mitosis)
        notice: protected environment here, haploid so must have viable essential alleles
     Note that female gametophyte is held in the ovule, protected by previous generation...

Anther Sac and Male Gametophyte Development
     Anther sac is a sporangium, is a part of the sporophyte
        makes microspores by meiosis
        this is a sexual reproductive act
     Microspores do mitosis to become a male gametophyte
        Microspore is covered by a cell wall that contains sporopollenin.
        Microspore does mitosis, to make two cells
            tube cell: will grow into a certain shape...
            generative cell: will do mitosis again to generate...
                two sperm cells, are actually in the cytosol of the tube cell!
     male gametophyte: is also called a pollen grain
        this is the organism that will engage in fertilization, as it makes the sperm
        Initially stays in the volume of the microspore cell wall, for travelling

Pollen and Pollination

        Anther sac ruptures, and releases pollen

                pollen grain has waxy covering, including sporopollenin...

        Pollen, being just a male, is NOT for dispersal,

        Pollen, being a male, has to deliver its sperm to an egg

Pollination is a distinct process, seen in most heterosporous species of plants

        Here pollination is movement of pollen from anther sac to stigma

                this is not dispersal, have not gotten to a new place where species was not

                this is also not fertilization, have not fused gametes yet...

                Pollination often involves mind games with animals, such as insects....

                        nectar or other food, perfumes that act as pheromones,

                        sepals and petals that look like potential mate...

        So pollination is a distinctive process from dispersal and from fertilization...

After pollination

        growth of pollen tube down style: some of the fastest known growing cells

                chemical signals sent between pollen and sporophytic tissue

                gets fed by sporophyte

                has to grow to egg in the megagametophyte, in ovule, in ovary...

                to deliver sperm to it

        Have we made gametes?   (yes)

        Is this male gametophyte a sexual reproductive organism? (yes)

Double Fertilization makes Zygote and Endosperm

        One Egg, Two Sperm.... of such arrangements are afternoon soap operas made...

        double fertilization

                entry of pollen tube into ovule through the open micropyle

                role of synergids to check sperm

                        sperm put into a synergid cell, into its actual cytosol!

                                sperm checked to see if right species, and of good "breeding"

                If of poor quality, sperm are killed

                If of good quality, one sperm (1N) fuses with egg (1N),

                        other fuses with central cell (N+N)

                double fertilization: produces 2N zygote, and 3N endosperm

Growth of Endosperm and Sporophytic Embryo

        Being triploid the endosperm grows fast

                fills with nutrient rich items in a liquid state to bath growing embryo,

                        this is the fluid in a coconut...

                Endosperm is a dead end, could not do meiosis at all well

                So endosperm will be absorbed by the sporpophytic embryo... very altruistic!

                      Sort of like you absorbing a sibling in the womb to help you grow....

        Zygote does mitosis, and becomes a young sporophyte = an embryo, which then grows

                endosperm expands first to surround embryo,

Embryo (2N) forms embryonic structures:

        formation of radicle, an embryonic root with a RAM

        cotyledon(s), specialized leaves

        SAM, will grow new leaves and stems

Note that the young sporophyte is surrounded by tissues of previous sporophyte

this embryo grows in ovule, ovule has integuments as an outer layer

        integuments of ovule form seed coat

        entire ovule is surrounded by the wall of the ovary for additional protection

Seeds and Fruits

Seed, a mature ovule

    Ovule is a part of the sporophytic generation

    Ovule is many things at many points in time. It:

        is a sporangium, makes a megaspore by meiosis

        is site of megagametophyte growth and development,

            the sporophyte feeds and protects this female gametophyte

        is site of double fertilization, when zygote and endosperm are formed

        protects and nurishes the growing embryo

        becomes a seed

    Seeds can go dormant for thousands of years, a major advantage over spores...

        problem of dispersal

            homosporous plants disperse spores, these travel on wind well

            seed plants have huge seeds, harder to disperse

Fruit

    ovary, with ovule(s) in it, matures into a fruit with seed(s) in it

    development of ovarian wall becomes the fruit coat

    issue of dispersal: various solutions,

        disperse by wind, modify fruit coat for dispersion

        often involving manipulation of animal behaviors

        disperse by animal help

            ovarian wall as food, poisons often placed in seeds

            or inner ovarian wall forms pit to protect seed

                and it passes through the digestive tract of the animal...

If Time: Slide Show

"With these obscene processes and prurient apparitions, the gentle and happy scholar of flowers has nothing to do."

        - John Ruskin, advice to students

        not probe too far into stamens and pistils -

Objectives:

Compare and contrast the life cycle of animals with that of flowering plants. Be able to indicate the ploidy and names of each stage in the life cycles, and to identify the sexual reproductive structures of flowering plants. Be sure that you can find and understand the roles of each of the following in the angiosperm life cycle: sporophytes, gametophytes, gametes, spores, sporangia, zygote, embryo. Be able to contrast homosporous and heterosporous plant life cycles.

Unlike animals where meiosis and fertilization are closely associated in the life cycle, plants separate these processes of sexual reproduction to different stages of the life cycle. Be sure you understand how both meiosis and fertilization are sexual and reproductive acts in the plant life cycle. In flowering plants what structures engage in the meiotic part of sexual reproduction, and what structures are involved in the fertilization part of sexual reproduction? Describe the process of double fertilization, including the roles played by the synergid and central cell. Understand the origin and function of the endosperm in the developing seed.

Be able to describe the parts of a perfectly complete flower and their major functions, versus that of an imperfectly incomplete flower.

Be able to describe how the angiosperm male gametophyte is formed, what cells it contains when mature, and the function(s) associated with it in the life of flowering plant species.

Correctly place and identify angiosperm ovule structures and functions it carries out at various stages of its development from it role as a sporangium to its being a seed.

Compare and contrast the functions of the ovarian wall when it first surrounds immature ovules, versus later when it surrounds mature seeds, in terms of what the ovarian wall might have to be doing at each of these different points of the life cycle.

Be able to describe various solutions that plants have evolved to address the problems of inbreeding, achieving pollination, fertilization, and dispersal of their species.

Figures 30.10, 38.3, 38.5 and 38.7 cover most of the structures and processes described in this lecture and may be good ones to review.

For review, see self quiz questions #1-3 of chapter 30, and questions #2, 3, and 4 of chapter 38.

Needed overheads and items:

Fig. 30.113e, (Campbell and Reece, 2005) orchid
Fig. 38.03ax1, (Campbell and Reece, 2005) lilly
187_K78_104b_Lilium_floral_bud_ls.jpg
178_K79_104d_Lilium_anthers_xs_late_prophase.jpg
181_K79_104e_Lilium_anthers_xs_metaphase_one.jpg
180_K79_104f_Lilium_anthers_xs_second_meiotic_division.jpg
182_L79_104g_Lilium_anther_xs_tetrads.jpg
183_K79_104h_Lilium_anthers_xs_microspores.jpg
184_K79_104i_Lilium_anthers_xs_pollen.jpg
Fig. 38.6, pollen
185_K81_132_Polygonatum_pollen_tubes_wm.jpg

187_K78_104b_Lilium_floral_bud_ls.jpg
188_K82_104a_Lilium_ovules.jpg
190_K82_104b3_Lilium_megasporocyte.jpg
191_K82_104c2_Lilium_megasporogenesis_anaphase_one.jpg
194_K82_104f3_Lilium_megaspores.jpg
198_K82_104k8_Lilium_mature_megagametophyte.jpg
199_K82_104l_Lilium_ovule_double_fertilization.jpg

157_K60_92a_Capsella_embryo_before_cotyledons.jpg
158_K60_92b_Capsella_embryo_early_cotyledons.jpg
159_K60_92c_Capsella_embryo_bending.jpg
160_K60_92d_Capsella_mature_embryo.jpg
Fig. 38.12, (Campbell and Reece, 2005) development pod

20581_violet_seedpod.jpg
171W3731_witch_hazel-capsules_dehiscence.jpg
Fig. 38.11, dispersion collage
171W3734_peach_drupe_section.jpg
Fig. 38.3, angiosperm gametogenesis

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky,
    R.B.Jackson- 2008-<u>Biology</u>. Eighth edition. Pgs. 252-253, 600-609, 618-621,
    625-634, 801-811. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition. Figures 30.113e, 38.3ax1, 38.12.
    Benjamin Cummings Press. San Francisco, CA.

Related issues:

Here are some studies of **fertilization** as done by flowering plants. One examines the spike in the cytosolic concentration of Ca^{+2} in the egg. Others look at the role of **synergids** in pollen tube recognition. Others have examined how **double fertilization** has evolved and why.

Escobar-Restrepo J-M., N. Huck, S. Kessler, V. Gagliardini, J. Gheyselinck, W-C. Yang, U. Grossniklaus- 2007-The FERONIA receptor-like kinase mediates male-female interactions during pollen tube reception- Science 317: (#5838, 8/3) 656-660

Faure J-E., C. Dumas- 2001-Fertilization in flowering plants. New approaches for an old story- Plant Physiology 125: 102-104

Ge L.L., H.Q. Tian, S.D. Russell- 2007-Calcium function and distribution during fertilization in angiosperms- American Journal of Botany 94: (#6) 1046-1060

Higashiyama T., H. Kuroiwa, S. Kawano, T. Kuroiwa- 2000-Explosive discharge of pollen tube contents in *Torenia fournieri*- Plant Physiology 122: 11-13

Kranz E., J. Kumlehn- 1999-Angiosperm fertilization, embryo and endosperm development- Plant Science 142: 183-197

McCormick S- 2007-Reproductive dialog- Science 317: (#5835, 8/3) 606-607

Double fertilization also occurs in some **gymnosperms**. Here is an article of one example.

Friedman W.E., J.S. Carmichael- 1996-Double fertilization in gnetales: Implications for understanding reproductive diversification among seed plants- International Journal of Plant Science 157: (#6, suppl.) S77-S94

Some angiosperm species have been found to have **sex chromosomes** but they are not of distinct sizes. Why that is has been an area of study.

Gerelick R- 2005-Theory for why dioecious plants have equal length sex chromosomes- American Journal of Botany- 92: (#6) 979-984

Ming R., J. Wang, P.H. Moore, A.H. Paterson- 2007-Sex chromosomes in flowering plants- American Journal of Botany 94: (#2) 141-150

In the ovule, **megasporogenesis** creates a spore, and it grows into a **female gametophyte**. Studies of these processes are ongoing.

Eriksen B., M. Fredrikson- 2000-Megagametophyte development in *Potentilla nivea* (Rosaceae) form northern Swedish Lapland- American Journal of Botany 87: (#5) 642-651

Jane W-N- 1997-Ultrastructure of the maturing egg apparatus in *Arundo formosana* Hack. (Poaceae)- International Journal of Plant Science 158: (#6) 713-726

How **microsporgenesis** results in spores and then **pollen grains** is considered in recent studies. Also how the **pollen tubes** grow and how they interact with the sporphytic tissues of the style is of interest.

Brown R.C., B.E. Lemmon- 2000-The cytoskeleton and polarization during pollen development in *Carex blanda* (Cyperaceae)- American Journal of Botany. 87: (#1) 1-11

Dixit R., J.B. Nasrallah- 2001-Recognizing self in the self-incompatibility response- Plant Physiology 125: 105-108

Obermeyer G., H. Klaushofer, M. Nagel, M. Höftberger, F.W. Bentrup- 1998-*In-vitro* germination and growth of lily pollen tubes is affected by protein phosphatase inhibitors- Planta 207: (#2) 303-312

Pasonen H-L., P. Pulkkinen, M. Käpyla- 2001-Do pollen donors with fastest-growing pollen tubes sire the best offspring in an amenophilous tree, *Betula pendula* (Betulaceae)?- American Journal of Botany 88: (#5) 854-860

Ressayre A., L. Dreyer, S. Triki-Teurtroy, A. Forchions, S. Nadot- 2005-Post-meiotic cytokinesis and pollen aperture pattern ontogeny: Comparison of development in four species differing in aperture pattern- American Journal of Botany 92: (#4) 576-583

Tanaka I., K. Ono, T. Fukuda- 1998-The developmental fate of angiosperm pollen is associated with a preferential decrease in the level of histone H1 in the vegetative nucleus- Planta 206: 561-569

Wilhelmi L.K., D. Preuss- 1997-Blazing new trails. Pollen tubes guidance in flowering plants- Plant Physiol. 113: 307-312

There have been studies of angiosperm **sperm** cells, and sperm cells in other plant species. There are reports of plant sperm contributing **paternal growth factors** that influence pattern formation during the growth of the embryo.

Bayer M., T. Nawy, C. Giglione, M. Galli, T. Meinnel, W. Lukowitz- 2009-Paternal control of embryonic patterning in *Arabidopsis thaliana*- Science 323: (#5930, 3/13) 1485-1488

Mogensen H.L., M.L. Rusche- 2000-Occurrence of plastids in rye (Poaceae) sperm cells- American Journal of Botany 87: (#8) 1189-1192

Southworth D., M. Cresti- 1997-Comparison of flagellated and nonflagellated sperm in plants- American Journal of Botany 84: (#9) 1301-1311

This study of **ovule** and **pollen growth** has truly reached new heights:

Kuang A., M.E. Musgrave, S.W. Matthews, D.B. Cummins, S.C. Tucker- 1995-Pollen and ovule development in *Arabidopsis thaliana* under spaceflight conditions- American Journal of Botany 82: (#5) 585-595

Here is an interesting review about the person who discovered the **alternation of generations** life cycle of plants.

Kaplan D.R., T.J. Cooke- 1996-The genius of Wilhelm Hofmeister: The origin of causal-analytical research in plant development- American Journal of Botany 83: (#12) 1647-1660

BIO 108   2010
Day 7, Lecture 18, Title: Plant Kingdom and Evolution.
Text Readings: Campbell et al. (2008), chapter 29 and pgs. 618-625.

Topics to cover:

    Before there were plants
    Advantages of living in the ocean versus on land: Three major issues
    Traits that evolve in response to selective pressures on land (disclaimer)
    Green algae, *Chara* sp.
    Bryophytes
    Ferns
    Gymnosperms
    Angiosperms
    Summary

We will consider the major traits, and the natural selection pressures that produced them, in plants that have helped make various groups of plants more adaptive for life on land. Characteristics found in mosses, ferns, pines, and flowering plants will be considered in response to selection for better dispersion, desiccation tolerance, and the need to protect vulnerable stages of their life cycle. How these changes have helped make plants adapted for life on land will be described.
    Fig. 30.5, bristlcone pine

Before there were plants
    about 600 mya, there were no plants on the planet, by 475 mya evidence of plants
    photosynthesis was done then by algae and bacteria
        Figure 28.19, Red Algae   Two red algae
        Figure 28.19, (Campbell and Reece, 2005) Kelp Forest
        Figure 28.21, Ulva, green alga
    Red, brown, and green algae
        note divided up the color spectra, evidence of competitive exclusion?
    Why no plant life on land?
        no ozone layer on early earth, needed one before life could go to land due to UV
        Why no ozone? Initially on earth the $[O_2]$ in atmosphere was low.
            once got above a certain point, started getting ozone layer
    In ocean
        Prime living space, as it never dries out
        Disperse single-celled gametes or zygotes all the time
    So why leave the oceans?
        Competition was strong for light, and for nutrients

Movement towards shore, for nutrients
promotes need for pigments to shade from UV, see secondary pigments...
Intertidal areas high in nutrients, also algae moved move up rivers to creeks
In these areas had to deal with periodic desiccation?
Red and Brown algae won the fight for the ocean
Green algae lost, and pushed towards shore and land....
Where do plants fit in eukaryotes?
Figure 26.21, EukaryotePhylogeny-L   Tree of eukaryotic diversity
note: animals, fungi, plants;
many other photosynthetic eukaryotes other than plants
most eukaryotic diversity is in the "protista"

Advantages of living in the ocean versus on land: Three major issues
Oceans as better environment, but tougher in terms of competition with other species...
Three major issues:
Dispersion: How to get to new locations.
in oceans this is via a water medium, can use single-celled items
Dealing with desiccation: A major issue on land,
in oceans this is not much of a problem
Protection of small, single-celled, stages: Always need to protect from herbivores,
On land also need to protect these stages from environmental stresses
that are not seen in the ocean...

Traits that evolve in response to selective pressures on land (disclaimer)
This is an over-simplification as do not have time for all the details
Take the course on plant evolution by Dr. Niklas for more information...
Traits that we will consider:
waxy secretions (cuticle, sporopollenin): obviously will resist water loss
vascular tissue: for moving water and organic matter in body of plant
alternation of generations: the type of life cycle seen in plants and others...
archegonia: a structure on the gametophyte that protects the egg, zygote,
and initially the young sporophyte
heterospory: different types of spores each will grow into different sexes
ovules/seeds: these will be used in dispersion
carpels and fruits: modifications of the sporophyte we see in flowers
We will use the following figure, and add features at the branch points
Figure 29-7-PlantEvolHighlts-NL
 no labels, plant phylogenetic tree

Groups we will consider:
        Charophytes, *Chara* sp. (a green alga),
                this is not a plant but an outgroup to the plant clade
        Bryophytes (moss)
        Pterophytes (ferns)
        Gymnosperms (pines)
        Angiosperms (flowering plants)

Green algae, *Chara* sp.
    Sister group to the plant clade, but not a plant
        Fig. 29.3, Charophyceans
        Figure 29.02x, Chara (Campbell etal., 2002)   shows chara with ruler
        lives in ponds, a freshwater alga
        Figure 17_K7_4a_chara_sex_organs_wm2   pseudoarchegonium
        antheridium makes sperm
        other structure shows where egg is made, lacks a true archegonium
        Fig. 13.6, sexual life cycle of algae
    Note that already have gametophyte generation and antheridia present...
    Zygote goes dormant, and is covered by waxy items in its cell wall
    Problem for dispersal
        zygote is release into water, and only one item to disperse at a time
        so in mud, which dries and holds it? Not the best for dispersal?
How to disperse better? Solution by a hypothetical ancestor plant...
    Note: the following would be needed to transition from Chara to a plant.
    Archegonium, if formed above water, would protect zygote, and get it to air
        note, Chara does not have a true archegonium
    Zygote does meiosis and makes four items to release, not one, if dispersed them
        then would have more items for dispersal?
        Figure 29.13, PlantAltGenHypoth-NL (Campbell etal., 2002)
        how sporophyte may have formed to make more spores...
        Do mitosis first, then even more spores made.
        Origin of sporophyte generation then as selection for better dispersal?
        Figure 13.6, AlternationGenerat-L   plant life cycle
    Already had gametophyte generation, and now have a sporophyte generation
    Archegonium: protects and feeds zygote and developing sporophyte
    Waxy cuticle: on spores, sporopollenin added to their cell walls
   So plants have an alternation of generation, create archegonia,
        cover their spores with sporopollenin
  Note: Dispersion: using spores for dispersion through air
      desiccation: archegonia protects single celled stage, sporopollenin protects spores
      supporting small stages: protecting egg, zygote, and spores

Bryophytes

So note how the above changes fit with the bryophytes

these are the most ancestral-like plants alive today

Figure 27244_moss_on_log   Moss on a log

do have alternation of generation life cycle, spores covered with sporopollenin, archegonia

Figure 29.8, PolytrichumLifeCycle-NL   moss life cycle

Fig. 29.16x2, Archegonium, (Campbell and Reece, 2002)   moss archegonia

Gametophytic adaptions: archegonium, the gametophyte feeds the sporophyte

Why didn't gametophyte elongate? and disperse eggs?

note, in air egg dispersion is awful, dry out too fast

disperse dried out eggs?? How would it get fertilized?

protection of fertilization event, single-stage/small structure

precludes use of gametes in dispersion

Figure 29.9, Sporophytes   Moss sporophytes

Sporophytic main purpose: make and disperse spores in air, not in water

an adaptation for dispersion that fits with life on land

makes spores in structures called sporangia

Spores covered with sporopollenin, note advantages of air vs. water dispersal

so want elongated spore producing stage, and make a lot of them...

for good dispersion see selection for height

As an asside, other eukaryotes use height for dispersion

Figure 28.30x1, Dictyostelium Collage (Campbell et al., 2002)

cellular slime mold,

height for dispersion of spores.

an example of convergent evolution, an analogous relationship...

So what we see in bryophytes

Figure 29.7, PlantEvolHightlits-NL   fill in parts with moss

Archegonium, alternation of generations, spores made in sporangia of sporophyte

spores covered by sporopollenin, etc...

(I disagree with the text where it claims that multicellular gametangia and apical meristems

are defining features of plants, as I know of examples of green algae that have these

traits...)

Ferns
Now consider changes that occur that lead to more complex plants. Ferns
        Figure 11161_northern_bracken_fern   sporophyte in woods
        Figure 29.13, Fern Life Cycle-NL
In this case sporophyte dominates the life cycle, how did this happen?
        Selection for longer sporophyte,
                have it do its own photosynthesis
                give it vascular tissue, allows it to be larger...
                Figure 29.11a, PolypodiumStem (Campbell et al., 2002) xylem and phloem
                        better for movement of items internally
                give it waxy cuticle
                        these last two mainly for dealing with desiccation resistance?
        Why select for larger sporophyte?
                Perhaps so it can make more spores, more sporangia
                        and so disperse better?
        General Fern Sori with indusia MC.jpg (Kowal et al., 1997)
        Fig. 29.13, fern life cycle, sorus with sporangia
        note how many sporangia are here, so much greater spore production...
Fern spore is small, covered with sporopollenin and disperses well in air
        General Fern Sori xs MC.jpg (Kowal et al., 1997)
        Figure 29.7, Fern Spore (Campbell et al., 2002)   fern spore whole mount
        Good for dispersal
If spore disperses somewhere, it may grow into a gametophyte
        Figure 29.23x5, Germinating   (Campbell et al., 2002) young fern gametophyte
        the gametophyte is hermaphroditic; this is a homosporous state
        homosporous: one type of spore, so grows into a hermaphroditic gametophyte
        hermaphroditic: a single individual makes both sperm and eggs
        good for dispersion, both sexes at once so no need to find a mate
        down side, inbreeding is high?
        General Fern gametophyte w sporo MC.jpg (Kowal et al., 1997)
But gametophyte is thin
        gametes, sperm and egg, need moisture
        so selection to reduce time spent in gametophyte generation
                this avoids desiccation
                and limits period of small parts to periods better suited to them
        So selection for smaller gametophyte, faster growing...
With vascular plants see several new traits
        Figure 29.7, PlantEvolHighlts-NL   fill in parts with fern
        vasculature (xylem and phloem) allows sporophyte to be large
                also see true stems, leaves and roots for the first time
        waxy cuticle now present on leaves and stems
        reduced gametophyte, dominant sporophyte

Gymnosperms
Fig. 30.5, Sequoia

First, a side step to heterospory and how it matters...

Figure 80_K29_28ab_Selaginella_Strobilus_ls   for heterosporous condition
Selaginella sporophylls MC.jpg (Kowal et al., 1997)
Selaginella megaspore & microspores MC.jpg (Kowal et al., 1997)

Note two sizes of spores, this is the Heterosporous condition

will have distinct spores each grows into just one sex
microspores grow into male gametophytes
megaspores grow into female gametophytes

Why?

Big spores do not disperse well, so seems to be a cost?
But large spores grow into large female gametophytes
and contain food, here see care of a small stage....?

Figure 30.6, PineLifeCycle-NL   pine life cycle

Heterospory is needed to make seeds, all seed plants are heterosporous

though not all heterosporous plants have seeds....

Consider this in pines, first ovulate cones

Figure 30.1, Pine Ovulate cones (Campbell et al., 2002)
Megaspores are no longer dispersed
so retain them in the sporangia
Ovulate cones are are made up of sporangia (i.e each ovule is one...)
Figure 97_K35_44ely_Pinus_young_ovulate_cone_ls   open ovulate cone
see the sporangia, this is a very young cone on the sporophyte
Figure 30.10, Pine Ovule (Campbell et al., 2002)   single bract with ovule
Retain megaspore in sporangium
In an ovule. This is excellant protection of a small stage!

These are not what we call a "pine cone", but they are microsporangiate cones

Figure 30.10, (Campbell et al., 2002) pine microsporangiate cones
sporangia here make microspores, and are released...
Figure 94_K35_44dl_Pinus_mature_microsporangiate_cone_ls   pine cone
Each sac is a sporangium, filled with microspores
These are released, to get to female cone
Figure 95_K35_44dps_Pinus_pollen   pine pollen
microspores grow into a male gametophyte
another name for the male gametophyte of a seed plant is pollen
Figure 29.23, Germinating (Campbell et al., 2002) young fern gametophyte
compare pollen of pine to gametophyte of fern. Which is bisexual?
one stays shut as released, other has to grow
so pine gametophyte is much less vulnerable to desiccation

Pollination is NOT fertilization

        Figure 30.6, PineLifeCycle-NL   pine life cycle

        Pollination as a new process, new selection pressures

            involves movement of male gametophyte, does not involve fertilization

        Figure 171W3710_red_pine_ovulate_cone   open pine cone

So spores are not dispersed in gymnosperms

        And pollen is not dispersed, it must do pollination. So what is used for dispersal?

Seeds are used for dispersion

        Figure 30.3, Pine Seed (Campbell et al., 2002)   to show dispersal

        Can go dormant, and sit in ground for decades!

        Can contain lots of food

        Large, so hard to disperse, but advantages outweigh this

        Figure 30.3, OvuleToSeed-NL   ovule, sporangium to seed

        So one major shift here is retention of megaspore

        Unisexual gametophytes promotes outbreeding

        fertilization in safe location, protection of sensitive stages

        early embryonic growth, again protection

So in shift to seed plants, like gymnosperms, see several new traits

        Figure 29.7, PlantEvolHeighlts-NL

        heterospory: microspores and megaspores produced in distinct sporangia

        ovules: retain megaspore, feeds growing female gametophyte

            is where fertilization occurs, where young sporophyte grows

            eventually develops into....

        seeds: are mature ovules, used for dispersion. So spores no longer are dispersed

Angiosperms

        Figure 20616_trout_lily   common spring plant

With pollen need pollination

        Gymnosperms mostly use wind pollination. Tend to repell animals..

        So do many angiosperms, but many involve animals

            a new factor for plants!

If attract pollinators,

        Figure 38.4   hummingbird and flower

        need to protect sensitive parts

        need to cover and hide ovules

Modifications to attract pollinators

        Figure 30.7   flower structures

        note carpel, ovarian wall

        protects where food and sensitive parts are

        Figure 30.14, CarpelOriginHypoth-NL (Campbell et al., 2002) sporophyll folding

        sporophyll folds over ovules, makes carpels/pistals

            so carpel is a new trait seen in flowering plants

        Figure 20582_rosehips_and_seeds   show edible parts

Problem of dispersal still here
> fruits derived from wall of carpel
> edible, or protective
> can allow for dispersion if can get animals to help, fine...

New traits seen in flowering plants include:
> Figure 29.7, PlantEvolHighlts-NL   fill in for angiosperms
>> carpel/fruit
>> flowers...

Summary
> Figure 29.7, PlantEvolHighlits-L
>> contrast with items I've mentioned
>> Note traits at each key branch point:
>>> archegonium, alt. of gen., sporopollenin on spores: Defines Plant Kingdom
>>> vasculature, reduced gametophyte,
>>>> waxy cuticle on shoots: Defines vascular plants
>>> heterospory, ovules, seeds: Defines seed plants
>>> flowers with carpels and fruits: Defines flowering plants
>> Note how functional issues are addressed
>>> for dispersal
>>> to resist dry conditions
>>> to protect vulnerable stages
>>> Many trade offs of the above.
>>>> no longer able to live well in the oceans...?

(Note: There are other groups of plants we have not considered, both living and extinct...)

Objectives:

Have an appreciation of the time frame of events in plant evolution. When did plants appear, and if there were no plants on the land earlier in the history of life on earth who did the photosynthesis? What were the major photosynthetic groups on earth before plants evolved? What are some major non-plant groups that do photosynthesis today?

You should know the fundamental characteristics that now are used to define the plant kingdom. (Comparing plants to an out group, such as some members of the green algae, might be helpful.)

The life cycle of plants should be clear to you, and given a representation of stages of the life cycles of mosses, ferns or pines you should be able to identify the stages in such a figure. Be sure that you could pick out in such a figure, and understand the roles of: sporophyte, gametophyte, gametes, zygote, spores, sporangia, archegonia and antheridia.

Be able to describe the influence of selection for dispersal and desiccation tolerance on the evolution of plants. What features of mosses, ferns, and pines promote their dispersal, or promote their avoidance of desiccation? Be able to identify the stages that are most sensitive to desiccation, and describe how each plant group copes with this problem. Be able to relate the ability of each of these plant groups to tolerate or avoid desiccation to the type of habitat where each is found. How do these plants differ in how they achieve dispersal?

Compare and contrast a homosporous versus a heterosporous life cycle in terms of the costs and benefits of each to a plant that has either trait. What is pollination, and how does it differ from dispersion and from fertilization?

The ovule, and how it develops into a seed, is a process you should be able to describe.

For review, see self-quiz questions #2, 3, 4, 6 and 8 of chapter 29, and questions #4-6 of chapter 30.

Needed figures and items:

Fig. 30.5, bristlcone pine
Figure 28.19, RedAlgae   Two red algae
Figure 28.19, Kelp Forest (Campbell and Reece, 2005)
Figure 28.21, Ulva, green alga
Figure 26.21, Eukaryote Phylogeny-L   Tree of eukaryotic diversity
Figure 29.7, PlantEvolHighlts-NL   no labels, plant phylogenetic tree
Fig. 29.3, Chara
Figure 29.2, Chara (Campbell and Reece, 2002)   shows chara with ruler
Figure 17_K7_4a_chara_sex_organs_wm2   shows antheridium and pseudoarchegonium
Fig. 13.6, sexual life cycle of algae
Figure 29.13, PlantAltGenHypoth-NL (Campbell and Reece, 2002)
Figure 13.6, AlternationGenerat-L   plant life cycle
Figure 27244_moss_on_log   Moss on a log
Figure 29.8, PolytrichumLifeCycle-NL   moss life cycle
Fig. 29.16, Archegonium (Campbell and Reece, 2002)   moss archegonia
Figure 29.9, Sporophytes   Moss sporophytes
Figure 28.30, DictyostelCollage (Campbell and Reece, 2002)
Figure 29.7, PlantEvolHightlits-NL   fill in parts with moss
Figure 11161_northern_bracken_fern   sporophyte in woods
Figure 29.13, FernLifeCyc-NL   fern life cycle
Figure 29.11, PolypodiumStem (Campbell and Reece, 2002)   xylem and phloem
General Fern Sori with indusia.jpg (Kowal et al., 1997)
Fig. 29.13, fern life cycle, sorus with sporangia
General Fern Sori xs.jpg (Kowal et al., 1997)
Figure 29.7, FernSpore (Campbell and Reece, 2002)   fern spore wholemount
Figure 29.23, Germinating (Campbell and Reece, 2002)   young fern gametophyte
General Fern gametophyte w sporo MC.jpg (Kowal et al., 1997)
Figure 29.7, PlantEvolHighlts-NL   fill in parts with fern
Fig. 30.5, Sequoia
Figure 80_K29_28ab_Selaginella_Strobilus_ls   for heterosporous condition
Selaginella sporophylls MC.jpg (Kowal et al., 1997)
Selaginella megaspore & microspores MC.jpg (Kowal et al., 1997)
Figure 30.6, PineLifeCycle-NL   pine life cycle
Figure 30.1, Pine ovulate cone (Campbell and Reece, 2002)   young cones of pine
Figure 97_K35_44ely_Pinus_young_ovulate_cone_ls   open cone
Figure 30.10, PineOvule (Campbell and Reece, 2002)   single bract with ovule
Figure 30.10, (Campbell and Reece, 2002)   pine microsporangiate cones
Figure 94_K35_44dl_Pinus_mature_microsporangiate_cone_ls   pine cone
Figure 95_K35_44dps_Pinus_pollen   pine pollen
Figure 29.23, Germinating (Campbell and Reece, 2002)   young fern gametophyte
Figure 30.6, PineLifeCycle-NL   pine life cycle
Figure 171W3710_red_pine_ovulate_cone   open pine cone
Figure 30.3, PineSeed (Campbell and Reece, 2002)   to show dispersal

Figure 30.3, OvuleToSeed-NL ovule, sporangium to seed
Figure 29.7, PlantEvolHeighlts-NL
Figure 20616_trout_lily common spring plant
Figure 38.4, hummingbird and flower
Figure 30.7, flower structures
Figure 30.14, CarpelOriginHypoth-NL (Campbell and Reece, 2002)
Figure 20582_rosehips_and_seeds show edible parts
Figure 29.7, PlantEvolHighlts-NL fill in for angiosperms
Figure 29.7, PlantEvolHighlits-L contrast with items I've mentioned

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Plant Diversity I: How plants colonized land. Chapter 29. And pages 618-625. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition. Fig. 28.19. Benjamin Cummings Press. San Francisco, CA.

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Kowal R.R., K.J. Sytsma, M.J. Ward, D.W. Woodland- 1997-Photo Atlas of the Vascular Plants- CD. Andrews University Press. Berrien Springs, Michigan.

Related issues:

These articles deal with aspects of the **evolution of seed plants**.

Fenster C.B., L.F. Galloway- 1997-Developmental homeostasis and floral form: Evolutionary consequences and genetic basis- International Journal of Plant Science 158: (#6S) S121-S130

Friedman W.E- 1996-Introduction to biology and evolution of the gnetales- International Journal of Plant Science 157: (#6, suppl.) S1-S2

Friis E.M., K.R. Pedersen, P.R. Crane- 2003-Origin and radiation of Angiosperms. Chap. 1.4.2, pgs. 97-102, in Palaeobiology II. D.E.G. Briggs and P.R. Crowther editors. Blackwell Pub. Oxford, U.K.

Herr J.M. jr-1995-The origin of the ovule- American Journal of Botany 82: (#4) 547-564

Kellogg E.A., J.L. Bennetzen- 2004-The evolution of nuclear genome structure in seed plants- American Journal of Botany 91: (#10) 1709-1725

Ricklefs R.E., S.S. Renner- 1994-Species richness within families of flowering plants- Evolution 48: (#5) 1619-1636

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Various features of **plant evolution** have been considered. Such as how the different **patterns of development** seen in different plant lineages came about. Or the role of **hybridization** in the production of new species of plants through reticulate evolution. How data from DNA sequences can be used to study plant phylogeny through quantitative trait loci (**QTL**) analysis. Also how plants are related to the various lineages of **algae**.

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Edwards D- 2003-Early land plants- Chap. 1.3.4, pgs. 63-66, in Palaeobiology II. D.E.G. Briggs and P.R. Crowther editors. Blackwell Pub. Oxford, U.K.

Ehrenreich I.M., M.D. Purugganan- 2006-The molecular genetic basis of plant adaptation- American Journal of Botany   93: (#7) 953-962

Friedman W.E., R.C. Moore, M.D. Puruggaman- 2004-The evolution of plant development- American Journal of Botany 91: (#10) 1726-1741

Gensel P.G., H.N. Andrews- 1987-The evolution of early land plants- American Scientist 75: 478-489

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Niklas, K.J- 1997-Tempos and patterns. Chapter 8 in The evolutionary biology of plants. University of Chicago Press, Chicago. 397 pgs.

Taylor T.N., E.L. Taylor- 1993-The biology and evolution of fossil plants. Prentice Hall, Englewood Cliffs, N.Y. 830 pgs.

Here are some more sources of information about **Bryophytes**; the mosses, liverworts, and hornworts.

Kroken S.B., L.E. Graham, M.E. Cook- 1996-Occurrence and evolutionary significance of resistant cell walls in Charophytes and Bryophytes- American Journal of Botany 83: (#10) 1241-1254

Shaw A.J- 2000-Population ecology, population genetics, and microevolution- Chapter 12, pgs. 369-402, in, <u>Bryophyte Biology</u>. A.J. Shaw and B.F. Goffinet editors. Cambridge University Press, Cambridge

This article reviews what is known of some of the earliest **vascular plants**, some of which were Rhyniophytes.

Hass H., T.N. Taylor, W. Remy- 1994-Fungi from the lower Devonian Rhynie chert: Mycoparasitism- American Journal of Botany 81: (#1) 29-37

Trewin N.H- 2003-The Rhynie chert. Chapter 3.4.5, pgs. 342-346, in <u>Palaeobiology II</u>. D.E.G. Briggs and P.R. Crowther editors. Blackwell Pub. Oxford, U.K.

Here is a nice article dealing with the uses of **ferns** in plant biology studies.

Hickok L.G., T.R. Warne, R.S. Fribourg- 1995-The biology of the fern *Ceratopteris* and its use as a model system- International Journal of Plant Science 156: (#3) 332-345

BIO 108   2010
Day 8, Lecture 19, Title: The Immune System.
Text Readings: Campbell et al. (2008), Chapter 43.

Topics to cover:

Innate Immunity
  Inflammation
  Complement System
  Natural Killer Cells (NK)
Acquired Immunity
  Humoral System (B cells)
    B cell Receptors and Clonal Selection
    Antibodies
    Primary and Secondary Immune Responses
  Cellular System (T cells)
    T Cell Receptor
    Some Cell Types
    Major Histocompatibility Complexes
    Role of Helper T Cells and Chemical Signaling

Will focus on basic systems for defense by mammals against disease
Note that there are fungal, protist, plant and bacterial defenses (Recall bacterial restriction enzymes?), analogous to what covered here, but we will not go into those...
In humans while the immune system is scattered it is made up of great numbers of cells.
About 2×10^{12} lymphocytes per human, which adds up to a mass equal to that of the brain!
  So immune system cells are a sort of "diffuse system"?

Innate Immunity
Non-adaptive defense systems, targets just some specific non-self structures or molecular shapes
  Fig. 43.2, overview of body defenses
  lysozyme, skin and secretions, cilia in trachea, etc...
  Includes physical barriers, hydrolytic enzymes, mechanical sweeping systems...
  While innate immunity can detect a wide range of specific non-self molecules
    can not change and adapt to changes in pathogens.
  Fig. 43.6, TLR system
  Works by receptors, Toll-like Receptors, on cell surface.
    detects non-self items, ex. peptidoglycan in bacterial cell walls...
  Fig. 43.3, phagocytosis
  Can also do phagocytosis of self and non-self items
    macrophages, neutrophils, and other cells take up cell debris

Inflammation

Fig. 43.8, inflammatory response

When stimulated by local cell damage basophils and mast cells release histamines

histamines enhance swelling and leakage of local blood vessels

Larger size promotes faster local blood flow, this raises local heat

Leakage swells local intercellular spaces, allows cells access

presses on pain receptors in skin, producing sensations of pain

pyrogens, from microbes and from leukocytes

raise local temperature, activates immune cells

vessel endothelial cells secrete chemokines (small positively charged peptides)

these attracts phagocytes; macrophages and dendritic cells

these all do phagocytosis of cell debris

are often killed by microbes, creating pus

Fig. 43.1, (Campbell and Reece, 2005) macrophage doing phagocytosis

The above cells can also travel to lymph nodes where they can interact with T cells

Fig. 43.7, lymphatic system

note lymph vessels, and lymph nodes

(What happens in the spleen and thymus?)

Complement System

Fig. 43.21, complement system

these are proteins secreted by liver cells into blood and lymph

they form a complex on non-self membranes

which forms pore in membrane, will lyse cell

formation of this complex is promoted by antibody binding

Natural Killer Cells (NK)

Detect proper display of surface proteins, including MHC I

If not displayed properly, kills the cell.

May detect cells becoming tumor cells, or cells that are infected.

Acquired Immunity

Specific defense systems, can change so adaptive,

all of these depend on some sort of molecule/molecule

interaction, require specific binding sites that must be made

These binding sites may be in secreted proteins or in proteins still in membranes of cells

Two major systems here Humoral and Cell-mediated systems.

Humoral System (B cells)

When activated these cells create and secrete proteins (antibodies) that bind up foreign molecules.

each antibody binding site is specific for ONE non-self molecular shape (epitope)

2

B cell receptors and Clonal Selection

How are these cells activated?

        Beta cells have B-cell receptors, are receptors displayed on surface of B cells

        Fig. 43.9, antigen receptors

        display about 10^5 receptors on surface of each cell for antigen

        These binding sites must bind only NON-SELF molecular shapes

            those B-cells that can bind SELF molecular shapes are killed in the thymus

Clonal selection

        Fig. 43.14, clonal selection

        "virgin" B cells are inactivated cells, display surface receptors

            each cell displays receptors that binds just one molecular shape

            different cells bind different molecular shapes

        Once activated, do mitosis and form memory and plasma cells

            Plasma cells pump out antibodies

            each plasma cell lives a few days-weeks secretes about 2000 antibodies per sec

                blood can have 20% Ig by dry mass!

            Memory cells display B-cell receptors and wait to be activated

            note membrane bound vs. free antibodies

                membrane spanning or secreted protein via change in RNA   processing

            Virgin B cells mature in bone marrow (or in liver when a fetus)

                Fig. 43.10, (Campbell and Reece, 2005) maturation of lymphocytes

            gene editting in B cell maturation and anitbody variety

                Fig. 43.13, immunoglobin maturation

                recombination of gene segments, and deletion of DNA occurs

                this is a case of differentiation associated with permanent changes in DNA

                after made and activated, this gene can undergo hypermutation

                    this promotes the diversity of the antibody binding sites made

Antibodies

Note conserved and variable parts of B cell receptor, and in antibody structure

        antibody types

            Fig. 43.20, antibody types

                (Focus on IgG and IgM, many subtypes of each class exist...)

            Ig = immunoglobin, note types

                IgM has many binding sites

                IgG and IgM are released into blood and lymph

                IgD is type used as B cell receptor

        So there is variability in types, thus Immunoglobins are part of a class of proteins

        genes coding for antibodies are part of an even larger superfamily of genes

            Fig. 24.71, (Alberts etal, 2002) Ig.jpg

            765 members of Ig family, diverged about 400 million yrs ago

            another example of gene duplication, note similar domains...

antigens

        Fig. 43.10, antigen variety

        one molecule can show several types of shapes over its surface,

        one molecule can induce many distinct antibodies...

        An antigen, induces antibodies against it

                it has epitopes in its structure, which are the regions the antibodies bind

antibody action on pathogens and toxins

        Fig. 43.21, antibody action

        Antibodies are proteins in blood, lymph, and secretions

                can get at exposed extracellular non-self items in the body

        covers microbes, reduces their mobility and binding abilities

        binds molecules, reduces their toxicity and binding sites

        Clumps bacteria and molecules, for digestion by phagocytotic cells

        conserved end of antibodies changes shape when antibody binds antigen

                This change in shape can be detected, promotes phagocytosis

Primary and Secondary Immune Responses

Primary immune response

        Fig. 43.15, primary and secondary immune response

        IgMs made mainly during primary response, have 10 binding sites each

                Fig. 43.20, Ig types

                hypermutation done to promote variation of Igs

                        mutation rate goes up a million fold!

Secondary immune response

        Fig. 43.15, primary and secondary immune response

        existing memory cells activated,

                more of them, so response is faster and greater, secrete mainly IgGs

        active vs. passive immunity

                can inject antibodies into someone, gives them passive immunity only

Cellular System (T cells)

        Fig. 23.4, (Alberts et al., 2002)

                note ER in B cell, not evident in T cell, one secretes alot other does not

        Fig. 43.12b, (Campbell and Reece, 2005) cytotoxic T cell

        Pathogens can avoid humoral system by entering host cells

                there the host cell's own plasma membrane hides internal pathogens

        So need a system to detect and eliminate cells that are infected

                this targets cells infected with viruses or bacteria, or cancerous cells...

T Cell Receptor
T cell receptors (TCRs) are used by T cells to examine items displayed on other cells
      Fig. 43.9, antigen receptors
            note is in Ig family, so similar to some domains of the receptor on B cells
      Display about 30000 TCRs on surface of each cell...
            each cell displays TCRs with just one type of binding site
            different T cells have different binding sites on TCR, so different specificity
      TCRs are membrane spanning proteins, with variable region, binding specific
      When in thymus T cells that make self-binding TCRs are killed
            cells not able to bind own MHCs are killed
            Fig. 43.10, (Campbell and Reece, 2005) lymphocyte development
            T cells mature in thymus

Some Cell Types
Some T cell types; involved in two very different systems when activated
      virgin T cells, have not encountered antigen when activated do cell division
      Cytotoxic T cells, induce apoptosis in infected cell, killing it
            Fig. 43.18, cytotoxic T cell
      Helper T cells, stimulate cell they are bound with, and secrete signals (cytokines)
            Fig. 43.17, T cell action
            Helper T cells bind to APC (antigen presenting cell) such as a macrophage
                  stimulates cytotoxic T cells and B cells
      [Suppressor T cells (not mentioned by text!) (also called regulatory T cells)
                loss of their functions leads to allergic responses, etc...]

Major Histocompatibility Complexes
T cell receptors must get a look at items in cell, can not enter cell,
      so depends on cell to display items found in it.
Two ways to do this.
      Might have an infection in the cell's cytosol, need to display these
            when T cells detect this will kill this cell to prevent pathogen reproduction
      Might have taken up foreign items by phagocytosis, need to diplay these
            cells that display antigens, but are not infected by them, are antigen presenter cells
            when T cells detect this will be activated to look at other cells in the area
      So want two different systems here, as do not want to kill APCs

Major histocompatibility complexes used to let T cells know which type of cell they are binding
      Fig. 43.12, T cells and MHCs
      MHC I, found on all nucleated cells of the body ("first the body" for #1)
      MHC II, found on immune system cells, including APCs
      Infected cells take parts of degraded molecules, for instance from proteosomes in cytosol,
            and import them into ER, where peptides are bound by MHC I
            veiscles go to plasma membrane and MHC I with antigen is displayed...
            parts of all degraded molecules are displayed, the vast majority are "self"
                but TCRs can not bind self-molecular shapes, so healthy cells are left alone
      APCs (macrophages, dendritic cells, some B cells) take up items by phagocytosis
            degrade items in lysosomes
            parts of degraded items fused to vesicles where MHC IIs are exposed to antigens
            vesicles go to plasma membrane and MHC II with antigen is displayed...
In addition have proteins that aid TCR/MHC interaction, CDs
      CD = "cluster of differentiation" cell-cell binding in cell differentiation and communication
            over 150 CDs are known so far....
      CD4 vs. CD8
      Fig. 43.18, Cytotoxic T cell action
            cytotoxic T cells use CD8 to help them bind MHC Is...
      Fig. 43.19 Helper T cell action
            helper T cells use CD4 to help them bind MHC IIs...

Role of Helper T Cells and Chemical Signaling
Any cell that is stressed can send out chemical signals (cytokines, histamines, interferons... etc...)
      these can help activate acquired immune system
Helper T cells can influence other systems in several ways...
      Once activated by binding with antigen displayed on APC can
            Fig. 43.17, Helper T cell action
            secrete cytokines
            bind with and activate B cells
Thus B cells can be activated by T cells, this allows cross talk between the two systems
            Fig. 43.19, T-dependent humoral response
      T-dependent antigens
            Fig. 49.13, (Campbell and Reece, 2002) T-dependent humoral response
            binding of helper T cell stimulates memory B cell activity
      vs. T-independent antigens
            antibodies against peptidoglycan, and other conserved pathogen features
            so no T cell activation needed
      Fig. 43.16, overview of specific immune response
      note how Helper T cells connect the two systems, other connections exist

Summary

All of this is cell signaling again, just different signals, receptors, and responses...
    One implication of this is that during infections we kill our own cells!
    Note how this imposes on cells the needs of the whole organism.
        So cells are now being subservient to the whole organism.

Innate system is fixed, but more ancient.
Adaptive system can work on extracellular or intracellular items.

Any multicellular organism must have a system to carry out similar functions...

Objectives:

Describe several mammalian non-specific defenses against infection. Which mechanisms depend on the direct action of cells and which are more indirect? What is involved in the inflammatory response? Describe the types of cells involved, the signals sent, and the responses made as a result of infection. What role do TLRs play? What is the complement system and what effect might it have on an infecting bacterium versus on an infecting virus?

What are the types of cells found in the B-cell system and what types are found in the T-cell system? Be able to describe each cell's function(s). How do these systems detect foreign items in the body, and where do they look? Be able to describe receptors used by each system, and their responses once activated. Describe how the immune system is kept from creating antibodies and T-cells that bind to self-antigens, or from destroying healthy cells. Be able to describe the roles of the lymphatic system, lymph nodes, and thymus in the immune system functions.

What is the structure of a typical antibody (IgG)? How is the type of antibody to be made determined and how is the DNA altered during this process? What is clonal selection, and what types of immune cells are involved in it or made by it? What effects can antibody binding have on a pathogen or a toxin? Be able to describe and contrast important features of the primary and secondary immune responses.

The interactions of MHCs and CD surface proteins and TCRs are important to know about so that the interactions of the T cell system can be understood. Which cell types have MHCI, and which MHCII? What cell types have CD4, and which have CD8? What is the purpose of having two parallel sets of these proteins? What do different types of T cells do as their major functions?

What is an antigen presenter cell and what role does it play in the activation of the immune system? What are cytokines and what role do they serve? Be sure you understand how the B-cell system and innate immune cells can interact with the T-cell system.

For review, see self-quiz questions #2, 3, 5 and 6 of chapter 43.

Needed overheads and items:

Fig. 43.2, overview of body defenses
Fig. 43.6, TLR system
Fig. 43.3, phagocytosis
Fig. 43.8, inflammatory response
Fig. 43.1, (Campbell and Reece, 2005) macrophage doing phagocytosis
Fig. 43.7, lymphatic system
Fig. 43.21, complement system
Fig. 43.9, antigen receptors
Fig. 43.14, clonal selection
Fig. 43.10, (Campbell and Reece, 2005) maturation of lymphocytes
Fig. 43.13, immunoglobin maturation
Fig. 43.20, antibody types
Fig. 24.71, (Alberts etal, 2002) Ig.jpg
Fig. 43.10, antigen variety
Fig. 43.21, antibody action
Fig. 43.15, primary and secondary immune response
Fig. 43.20, Ig types
Fig. 43.15, primary and secondary immune response
Fig. 23.4, (Alberts et al., 2002)
Fig. 43.12b, (Campbell and Reece, 2005) cytotoxic T cell
Fig. 43.9, antigen receptors
Fig. 43.10, (Campbell and Reece, 2005) lymphocyte development
Fig. 43.18 cytotoxic T cell
Fig. 43.17, T cell action
Fig. 43.12, T cells and MHCs
Fig. 43.18, Cytotoxic T cell action
Fig. 43.19 Helper T cell action
Fig. 43.17, Helper T cell action
Fig. 43.19, B cell activation
Fig. 43.13, (Campbell and Reece, 2002) T-dependent humoral response
Fig. 43.16, overview of specific immune response

References:

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walker- 2002-The adaptive immune system. Chapter 24, pgs. 1363-1421, Figure 24.71, in <u>Molecular Biology of the Cell</u>. 4th edition. Garland Science, N.Y., N.Y.

Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walker- 2002-Pathogens, infection, and innate immunity. Chapter 25, pgs. 1423-1463, in <u>Molecular Biology of the Cell</u>. 4th edition. Garland Science, N.Y., N.Y.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-The Immune System. Chapter 43. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition, Figures 43.1, 43.10, 43.12b. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figures 43.13. Benjamin Cummings Press. San Francisco, CA.

Related issues:

For some nice reviews on how the **immune system** functions, especially to deal with viral infections, or how immunology relates to cell biology see:

> Flint S.J., L.W. Enquist, V.R. Racaniello, A.M. Skalka- 2004-Viral offense meets host defense, chapter 15, pgs. 530-594, in <u>Principles of Virology: Molecular biology, pathogenesis, and control of animal viruses</u>- ASM Press. Washington D.C.
> Mellman I- 2007-Private lives: Reflections and challenges in understanding the cell biology of the immune system- Science 317: (#5838, 8/3) 625-627

Here is an article that examines how **long term immune memory** is achieved.

> Ahmed R., D. Gray- 1996-Immunological memory and protective immunity: Understanding their relation- Science 272: (#5258, Apr. 5) 54-60

Extracellularly, the **complement system** are proteins that act to puncture the membranes of foreign cells. Some pathogens use this system to escape from cells they have infected.

> Hadders M.A., D.X. Beringer, P. Gros- 2007-Structure of C8α-MACPF reveals mechanism of membrane attack in complement immune defense- Science 317: (#5844, 9/14) 1552-1554
> Kafsack B.F.C., J.D.D. Pena, I. Coppens, S. Ravindran, J.C. Boothroyd, V.B. Carruthers- 2008-Rapid membrane disruption by a perforin-like protein facilitates parasites exit from host cells- Science 323: (#5913, 1/23) 530-533

The **thymus** and other parts of the immune system play a role in eliminating any self-reactive B cells or T cells.

> Gardner J.M., J.J. DeVoss, R.S. Friedman, D.J. Wong, Y.X. Tan, X. Zhou, K.P. Johannes, M.A. Su, H.Y. Chang, M.F. Krummel, M.S. Anderson- 2008-Deletional tolerance mediated by extrathymic *aire*-expressing cells- Science 321: (#5890, 8/8) 843-847
> Kyewski B- 2008-A breath of Aire for the periphery- Science 321: (#5890, 8/8) 776-777
> Zachariah M.A., J.G. Cyster- 2010-Neural crest-derived pericytes promote egress of mature thymocytes at the corticomedullary junction- Science 328: (#5982, 5/28) 1129-1135

One mechanism of the innate immune system is to use **miRNA** (i.e. **RNA interference**) to block the prodution of viruses in an infected cell.

> Lanford R.E., E.S. Hildebrandt-Eriksen, A. Petri, R. Persson, M. Lindow, M.E. Munk, S. Kauppinen, H. Ørum- 2010-Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection- Science 327: (#5962, 1/8) 198-201

Here are a few articles dealing with aspects of the **T cell system**. Including how T cells get selectec, how they develop, and studies of how they move and interact.

Bannard O., M. Kraman, D.T. Fearon- 2009-Secondary replicative function of CD8^{+} T cells that had developed an effector phenotype- Science 323: (#5913, 1/23) 505-509

Coffman R.L- 2010-The origin of T_H2 responses- Science 328: (#5982, 5/28) 1116-1117

Feau S., S.P. Schoenberger- 2009-*Ex Uno Plura*- Science 323: (#5913, 1/23) 466-467

Huang G.N., D.L. Huso, S. Bouyain, J. Tu, K.A. McCorkell, M.J. May, Y. Zhu, M. Lutz, S Collins, M. Dehoff, S. Kang, K. Whartenby, J. Powell, D. Leahy, P.F. Worley- 2008-NFAT binding and regulation of T cell activation by the cytoplasmic scaffolding Homer proteins- Science 319: (#5862, 1/25) 476-481

Khanna K.M., J.T. McNamara, L. Lefraçois- 2007-*In situ* imaging of the endogenous CD8 T cell response to infection- Science 318: (#5847, 10/5) 116-120

Oberdoerffer S., L.F. Moita, D. Neems, R.P. Freitas, N. Hacoben, A. Rao- 2008-Regulation of CD45 alternative splicing by heterogeneous ribonucleoprotein, hnRNPLL- Science 321: (#5889, 8/1) 686-691

O'Shea J.J., W.E. Paul- 2010-Mechanisms underlying lineage commitment and plasticity of helper CD4^{+} T cells- Science 327: (#5969, 2/26) 1098-1102

Reiner S.L., F. Sallusto, A. Lanzavacchia- 2007-Division of labor with a work force of one: Challenges in specifying effector and memory T cell fate- Science 317; (#5838, 8/3) 622-625

Setoguchi R., M. Tachibana, Y. Naoe, S. Muroi, K. Akiyama, C. Tezuka, T. Okuda, K. Taniuchi- 2008-Repression of the tanscription factor Th-POK by Runx complexes in cytotoxic T cell development- Science 319: (#5864, 2/8) 822-825

Teixeiro E., M.A. Daniels, S.E. Hamilton, A.G. Schrum, R. Bragado, S.C. Jameson, E. Palmer- 2009-Different T cell receptor signals determine CD8^{+} memory versus effector development- Science 323: (#5913, 1/23) 502-505

van Heijst J.W.J., C. Gerlach, E. Swart, D. Sie, C. Nunes-Alves, R.M. Kerkhoven, R. Arens, M. Correia-Neves, K. Schepers, T.N.M. Schumacher- 2009-Recruitment of antigen-specific CD8^{+} T cells in response to infection is markedly efficient- Science 325: (#5945, 9/4) 1265-1269

Wakim L.M., J. Waithman, N. van Rooijen, W.R. Heath, F.R. Carbone- 2008-Dendritic cell-induced memory T cell activation in nonlymphoid tissues- Science 319: (#5860, 1/11) 198-202

Wang B., T.M. Primeau, N. Myers, H.W. Rohrs, M.L. Gross, L. Lybarger, T.H. Hansen, J.M. Connolly- 2009-A single peptide-MHC complex positively selects a diverse and specific CD8 T cell repertoire- Science 326: (#5954, 11/6) 871-874

The immune system's regulation involves **suppression**. This is carried out by various cell types, and can be used by cancer cells to block immune attacks on them.

Annacker O., R. Pimenta-Araujo, O. Burlen-Befranoux, A. Bandeira- 2001- On the ontogeny and physiology of regulatory T cells- Immunological Reviews 182: 5-17

Chatenoud L., B. Salomon, J.A. Bluestone- 2001-Suppressor T cells- They're back and critical for regulation of autoimmunity!- Immunological Reviews 182: 149-163

Chaudhry A., D. Rudra, P. Trenting, R.M. Samstein, Y. Liang, A. Kas, A.Y. Rudensky- 2009-CD4$^+$ regulatory T cells control T$_{H17}$ responses in a STAT3-dependent manner- Science 326: (#5955, 11/13) 986-991

Levings M.K., R. Sangregorio, C. Sartirana, A.L. Moschin, M. Battaglia, P.C. Orban, M-G. Roncarolo- 2002-Human CD25$^+$ CD4$^+$ T suppressor cell clones produce transforming growth factor β but not interleukin 10, and are distinct from type 1 T regulatory cells- Journal of Experimental Medicine 196: (10) 1335-1346

Marx J- 2008-Cancer's bulwark against immune attack: MDS cells- Science 319: (#5860, 1/11) 154-156

Melton L- 2002-Subduing suppressors. Silencing certain immune cells could defeat disease- Scientific American 287: (#6) 28-29

Mold J.E., J. Michgëlsson, T.D. Burt, M.O. Muench, K.P. Beckerman, M.P. Busch, T-H. Lee, D.F. Nixon, J.M. McCune- 2008-Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells *in utero*- Science 322: (#5907, 12/5) 1562-1565

Shevach E.M., R.S. McHugh, C.A. Piccirillo, A.M. Thornton- 2001-Control of T-cell activation by CD4$^+$ CD25$^+$ suppressor T cells- Immunological Reviews 182: 58-67

Sakaguchi S., F. Powrie- 2007-Emerging challenges in regulatory T cell function and biology- Science 317: (#5838, 8/3) 627-629

These articles describe different systems for **antigen presentation** on various types of MHCs, and aspects of **antigen presenter cells** (APC).

Burgdorf S., A. Kautz, V. Böhnert, P.A. Knolle, C. Kurts- 2007-Distinct pathways of antigen uptake and intracellular routing in CD4 and CD8 T cell activation- Science 316: (4/27) 612-616

Dudziak D., A.O. Kamphorst, G.F. Heidkamp, V.R. Buchholz, C. Trumpfheller, S. Yamazaki, C. Cheong, K. Liu, H-W. Lee, C.G. Park, R.M. Steinman, M.C. Nussenzweig- 2007-Differential antigen processing by dendritic cell subsets *in vivo*- Science 315: (1/5) 107-111

Lukacs-Kornek V., S.J. Turley- 2008-Chaperone puts the brakes on- Science 322: (#5908, 12/12) 1640-1641

The **innate immune system** includes many cell types and many functions. One function is to stimulate the production of proteins that bind up essential minerals, so that infecting microbes are stared for them and grow less rapidly. They often use Toll-like receptors to detect the presence of pathogens, and detection often results in interferon release to stimulate other responses. At the intracellular level, it has been found that some pathogen RNAs, or DNAs, can be detected by host cell factors. So there are many types of receptors for pathogen associated molecules.

Geissmann F., M.G. Manz, S. Jung, M.H. Sieweke, M. Merad, K. Ley- 2010-Development of monocytes, macrophages, and dendritic cells- Science 327: (#5966, 2/5) 656-661

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Here is a neat article that notes roles of **platelets** as part of our the innate immune system, and how **neutrophils**, use extracellular strands of DNA to catch bacteria!

Leslie M- 2010-Beyond clotting: The powers of platelets- Science 328: (#5978, 4/30) 562-564

There are reports of entirely **new classes of receptors** used by the **innate immune system** to detect pathogenic molecules. Here are a few of these.

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Ting J.P.Y., J.A. Ducan, Y. Lei- 2010-How the noninflammasome NLRs function in the innate immune system- Science 327: (#5963, 1/15) 286-290

Vijay-Kumar M., J.D. Aitken, F.A. Carvalho, T.C. Cullender, S. Mwangi, S. Srinivasan, S.V. Sitaraman, R. Knight, R.E. Ley, A.T. Gewirtz- 2010-Metabolic syndrome and altered gut microbiota in mice lacking toll-like receptor 5- Science 328: (#5975, 4/9) 228-231

The immune system is a two-edged sword. It can be evaded or turned against us. This can produce **autoimmune illnesses** and in some cases **tumor cells** induce these errors. Another article describes how the **tuberculosis** microbe uses our immune system to protect itself.

Russell D.G., C.E. Barry 3rd, J.L. Flynn- 2010-Tuberculosis: What we don't know can, and does, hurt us- Science 328: (#5980, 5/14) 852-856

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Zindl C.L., D.D. Chaplin- 2010-Tumor immune evasion- Science 328: (#5979, 5/7) 697-698

Making an effective **vaccine** that stimulates the immune system properly is not easy. There have been many setbacks in attempts to make an anti-HIV vaccine, for instance. Others are looking into uses of rare combinations of codon usages to make a weak strain of polio virus that could then be used as a live attenuated vaccine. Another article looks at attempts to make vaccines against malaria.

Coleman J.R., D. Papamichail, S. Skiena, B. Futcher, E. Wimmer, S. Mueller- 2008-Virus attenuation by genome-scale changes in codon pair bias- Science 320: (#5884, 6/27) 1784-1787

Enserink M- 2008-"Biased" viruses suggest new vaccine strategy for polio and other diseases- Science 320: (#5884, 6/27) 1709

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Moore J.P., P.J. Klasse, M.J. Dolan, S.K. Akuja- 2008-A step into darkness or light?- Science 320: (#5877, 5/9) 753-755

Vogel G- 2010-The "Do unto others" malaria vaccine- Science 328: (#5980, 5/14) 847-848

Watkins D.I- 2008-The vaccine search goes on- Scientific American 299: (#5, Nov.) 69-76

Here are some articles dealing with aspects of the **B cell system**. Including how their cells develop in germinal centers, and their role in autoimmune diseases. Another describes how antibodies can swap subunits to make new combinations. After activation, some B cells under hypermutation to vary the genes for antibody production.

Allen C.D.C., T. Okada, H.L. Tang, J.G. Cyster- 2007-Imaging of germinal center selection events during affinity maturation- Science 315: (1/26) 528-531

Bankovich A.J., S. Raunser, Z.S. Juo, T. Walz, M.M. Davis, K.C. Garcia- 2007-Structural insight into Pre-B cell receptor function- Science 316: (4/13) 291-294

Burton D.R., I.A. Wilson- 2007-Square-dancing antibodies- Science 317: (#5844, 9/14) 1507-1508

Keenan R.A., A. DeRiva, B. Corleis, L. Hepburn, S. Licence, T.H. Winkler, I-L. Mårtensson- 2008-Censoring of autoreactive B cell development by the pre-B cell receptor- Science 321: (#5889, 8/1) 696-699

Matthews R- 2007-The B cell slayer- Science 318; (#5854, 11/23) 1232-1233

Notkins A.L- New predictors of disease- Scientific American 296: (#3, March) 72-79

Unniraman S., D.G. Schatz- 2007-Strand-biased spreading of mutations during somatic hypermutation- Science 317: (#5842, 8/31) 1227-1230

Van der NeutKolfschaten M., J. Schuurman, M. Losen, W.K. Bleeker, P. Martínez-Martínez, E. Vermeulen, T.H. den Bleker, L. Wiegman, T. Vink, L.A. Aarden, M.H. DeBaets, J.G.J. van de Winkel, R.C. Aulberse, P.W.H.I. Parren- 2007-Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchanges- Science 317: (#5844, 9/14) 1514-1557

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Here are some articles that examine aspects of the **inflammatory response** and its role in the immune system.

Boilard E., P.A. Nigrovic, K. Larabee, G.F.M. Watts, J.S. Coblyn, M.E. Weinblatt, E.M. Massurotti, E. Remold-O'Donnell, R.W. Farndale, J. Ware, D.M. Lee- 2010- Platelets amplify inflammation in arthritis via collagen-dependnet microparticle production- Science 327: (#5965, 1/29) 580-583

Choi E.Y., E. Chavakis, M.A. Czabanka, H.F. Langer, L. Fraemohs, M. Economopoulou, R.K. Kundu, A Orlandi, Y.Y. Zheng, D.A. Prieto, C.M. Ballantyne, S.L. Constant, W.C. Aird, T. Papayannopoulou, C.G. Gahmberg, C. Weber, T. Chavakis- 2008- Del-1 an endogenous leukocyte-endothelial adhesion inhibitor, limits inflammatory cell recruitment- Science 322: (#5903, 11/14) 1101-1104

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And **non-animals** have **innate immune systems** as well.

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BIO 108   2010
Day 8, Lecture 20, Title: Animal Development I.
Text Readings: Campbell et al. (2008), pgs. 1025-1044.

Topics to cover:

> **Frog Development**
> > **Body Axes and Early Cleavage**
> > **Gastrulation and Early Tissues**
> > **Neurulation**
> > **Positional Information**
> **Chicken Development**
> > **Limb Bud Induction**
> **Human Early Development**
> **Summary**

First will cover classical morphogenesis, then in next lecture will tie this to genetic controls.
Overview stages of animal development
> Zygote, morula, blastula, gastrula, neurula... etc...
Will compare these stages for frog, chicken, human (obviously animal development varies)
> A nice contrast of one non-amniote versus two amniotic species.
> A contrast of sources of food for embryo, from yolk versus placenta.
Will consider how developmental stages are induced/organized by signals from certain regions.
> The concepts of an organizer region and gradients of morphogens have wide application

Frog Development
> Frog as a model. Easy to study, external fertilization, rapid development

> **Body Axes and Early Cleavage**
> Early cleavage (to blastula)
> > Fig. 47.7, body axes in frog embryo
> > How did animal/vegetal poles get made?
> > > not just pigmentation difference here
> > > also mRNA, ribosomes, proteins, etc... mostly maternal factors
> > > > from maternal nurse cells back in follicle in ovary...
> > Note rotation of cortical cytosol with sperm entry creates gray crescent
> > Can tell anterior/posterior with animal/vegetal pole
> > Can tell dorsal/ventral with point of sperm entry and gray crescnt
> > Fig. 47.8, frog cleavage
> > > uneven cleavage, due to yolk-filled areas dividing slower
> > Morula
> > > a multicelled stage, no distinct internal cavity yet

Blastula
  now see an internal cavity, with distinct composition
  Blastocoel
    "coel" = space or internal cavity (Can students name one in us?)
    depends on cell-cell junctions between boundary cells
    advantage for SA/volume ratio?
    control of internal space? Two tissue layers here.
  Recall that definition of animal kingdom includes:
    development through a blastula stage,
    beyond this point see much more variability amongst species

Gastrulation and Early Tissues
Gastrulation involves infolding and cell movement
  Fig. 47.10, frog gastrulation
  note
    blastopore (relative to gray cresent)
      in this case opening by blastopore becomes annus (a deuterostome)
    archenteron (primitive gut) is begining of digestive tract
    destruction of blastocoel occurs during this process
    migration of cells
      note role of surface receptors/binders
        several types of cell adhesion molecules (CAMs) involved
    formation of yolk plug
At end of gastrulation have three layers of undifferentiated tissues (germ tissues)
  and one organ system begun (digestive tract), not completely developed yet...
Determination of cell fate begun
  Fig. 47.14, three embryonic germ layers in vertebrates
  Three tissue layers
    ectoderm, makes nervous system and organs derived from it, skin
    mesoderm, makes notochord, muscles, etc...
    endoderm, makes epithelial linings, pancreas, and other organs
    note, these are tissues, no mature organs yet at gastrula stage

Neurulation
This process begins formation of another organ system, central nervous system
  Fig. 47.12, neurulation and frog organogenesis
  Infolding above notochord, at dorsal side of dorsal/ventral axis
    it acts as an organizing center for this folding
  note
    neural folds
    neural tube, will lead to central nervous system (CNS)
    neural crest, cells in this lead to lead to peripheral nervous system (PNS)

Fig. 47.17, cellular shape and morphogenesis
　　　　role of cytoskeleton, connected to cadherins connecting two cells
　　　　　　also fibronectins and extracellular matrix play a role
Fig. 47.19, cadherins
note how loss of cadherin (a type of cell adhesion molecule) alters blastula

Positional Information
　　　　Fig. 47.12, neurulation and frog organogenesis
　　　　Note that later get more anterior/posterior changes
　　　　　　leading to somites, will become vertebrae
　　　　　　Information along anterior/posterior gives borders of somites
　　　　　　　　and influences determination of what each somite forms
　　　　　　Also uses dorsal/ventral information in each somite...
　　　　But are these influences produced by extracellular or intracelluar signaling?
　　　　　　See both used in various situations
　　　　Fig. 47.21, frog fate map
　　　　　　Note can go back to blastula and identify the cells that will lead to organs
　　　　　　In this case can inject a dye into cell and follow where it ends up...
　　　　Fig. 21.5, (Alberts etal, 2002) frogcellfate.jpg
　　　　　　Can insert various probes into separate cells
　　　　　　can detect over time.
　　　　　　assumes these probes are not swapped
　　　　　　　　between cells, and not diluted out
　　　　Differentiation of cells involves their changing into "mature" form
　　　　　　distinct from determination of future fate,
　　　　　　　　as fate can be fixed even if not yet differentiated...
　　　　Fig. 47.23, cytoplasmic determinants in frog
　　　　　　Items in cytosol are not evenly distributed here
　　　　　　Example, note role of items associated with the gray crescent
　　　　　　If divide zygote unevenly so gray crescent not shared, get odd growth
　　　　　　If divide evenly get two normal embryos.... just a bit smaller.
　　　　So items in cytosol can, by their uneven distribution, help determine fate of cells
　　　　　　this is an example of intracellular factors influencing development
　　　　In other cases signals are secreted, see this in dorsal lip of blastopore
　　　　Fig. 47.25, newt "organizer" region
　　　　　　regions that secrete morphogens, and influence pattern formation
　　　　　　　　are called organizing regions
　　　　　　　　the dorsal lip of the blastopore is an organizing region
　　　　　　role of dorsal lip of blastopore seen in transplant experiment
　　　　　　　　sends out signal, extracellular, that influences surrounding cells
(movie, 21_1.mov, frog development, MBOTC)

Chicken Development
Chicken as a model, an amniote, external growth, easy to obtain...
 Blastula
   Fig. 47.12, (Campbell and Reece,2002) chick blastula/gastrulation
   a very flattened blastula
   huge yolk, such uneven cleavage that yolk is kept intact and out of embryo
    note how this differs from frog where yolk ends up in the embryo
 Gastrulation
   Creates three tissue layers
   here blastopore is called a primitive streak, but still where annus forms
 Neurulation
   Will infold to form neural tube that will become the CNS
 Extra embryonic membranes and amnionic egg
   Fig. 47.17, (Campbell and Reece, 2005) chick extraembryonic membranes
   embryo
   extraembryonic structures here are "membranes"
    these are tissues, not cell membranes... various functions
    amnion, dessication prevention and cushioning of embryo
    yolk, food source
    allantois, waste and gas exchange
    chorion, cushioning and waste and gas exchange
   Fig. 40.17 (Purves et al., 1998) chick_membrane_development.jpg
   note how membranes wrap around, yolk, embryo, etc...
 Implications of extraembryonic membranes:
   Yolk can be larger than would fit in embryo, larger than diffusion limits...
    So more food can be stored for embryo's use.
   Protection of embryo allows survival in more challenging environments
    Consider the shape of the egg, helps or hinders water loss to the outside?
   Gas exchange is promoted by the membranes
    this is important since embryo becomes bigger
    often has a high metabolic rate
    and surface SA/VOL is poor...

Limb Bud Induction
Positional information in limb growth in chicken development
   Fig. 47.25, Vertebrate limb development
Here see organizing regions that secrete chemical signals (i.e. morphogens)
   (recall dorsal lip of blastopore...)
   AER = apical ectodermal ridge, secretes signals that induce and organize
    growth proximal/distal axis of limb
   ZPA = zone of polarizing activity, secretes signals that induce and organize
    anterior/posterior organization of limb
    this sets finger/bone orientation

Fig. 21.13, (Alberts etal, 2002) chicklimb.jpg

Fig. 47.26, pattern formation and ZPA

transplant experiment, if add an extra ZPA region alters development

note how similar are:

ZPA and dorsal lip of blastopore, both are organizing regions

organizing region sets up a concentration gradient of signal

These are called "morphogens," and are proteins or other types of organic matter

For ZPA the signal is a protein called sonic hedgehog

cells with receptors sense the signal and have fate altered

Human Early Development

Blastula/blastocyst, in mammals it is called a blastocyst as has distinct parts

Fig. 47.16, human embryogenesis

note in this figure part (1)

blastula/blastocyst

blastocoel, as you would expect in a blastula this is a body cavity...

inner cell mass, this will create embryonic and extraembryonic cells

trophoblast, will make extraembryonic items only

note in this figure part (2)

inner cell mass becomes epiblast and hypoblast

Gastrulation

note in this figure part (4)

epiblast, forms ectoderm and mesoderm and endoderm

so now have all three germ layers of the embryo

The extraembryonic membranes have their main origins from the following layers

(some other cells also contribute across these layers...)

amnion, from epiblast

chorion, from tropoblast

yolk sac, from hypoblast

allantois, from hypoblast and tropoblast

The chorion and allantois and yolk sac make up the fetal side of the placenta.

items can cross the placenta (both proteins and cells!)

high surface area

Other views of mammalian embryonic development

Fig. 55.12 (Raven and Johnson, 1995) mammalian_gastrulation.jpg

shows a better view of gastrulation, note primitive streak

Fig. 3.6 (Marieb and Mallatt, 1997) human_blastopore

note positional information. We are deuterostomes, First the anus!

Like in chicken, mammals have gastrulation and form yolk sac around yolk

Fig. 3.11, (Marieb and Mallatt, 1997) Human_4week.jpg

extra-embryonic membranes in humans

Fig. 40.18, (Purves et al., 1998) human_40days.jpg

5

Summary

Fig 5_6 Patterns of Chordate Cleavage (Hidebrand and Goslow, 2001)

Note similarities of chick embryogenesis and human

    Both have an initially flattened blastula, both form four extra-embryonic membranes.

    Both use extraembryonic membranes to get items from outside of embryo to deliver to
        embryo.

Note use of signals to induce stages of development

    Cytosolic signals, often maternally derived, divided unevenly help determine cell fate

    Extracellular signals (morphogens), secreted by organizing regions can induce cell fates

(If time show movie 21_4, of fish gastrulation; MBOTC)

Objectives:

We have three model vertebrates to consider: frogs, chickens, and humans. Be able to compare them to each other at the early stages of embryogenesis and contrast the patterns they show as they carry out gastrulation and neurulation.

Given a frog embryo be able to identify the early anterior/posterior axis and the dorsal/ventral axis of the embryo that will grow from it.

Describe how experiments that divide up a frog zygote, and experiments that track the developmental fate of early cells, are consistent with the presence of cytosolic factors. Be able to describe the use of transplantation experiments to study embryonic organizing centers. What are examples of organizing centers and how is it proposed that they operate: What molecular systems are necessary?

At what point in animal development are there distinct tissues present, and what are their names? What is the difference between determination of cell fate and differentiation of cell type? At what point do the first organs begin to form?

The frog embryo has lots of yolk. Where does the yolk end up in the gastrula and neurula stages of a frog? Where does the yolk end up in similar stages of the chicken embryo? How does the amount of yolk influence early cleavage and the shape of the blastula? How do human embryos obtain nutrients for growth? In each of these three vertebrates how does the embryo manage to gain access to food whether in the yolk or not?

Know the structures in the amniotic egg, and be able to compare the function and role of these membranes in chickens versus humans (figs. 47.13, 47.15, and 47.16 may be helpful here).

For review, see self-quiz questions #3-8 of chapter 47.

Needed overheads and items:

Fig. 47.7, body axes in frog embryo
Fig. 47.8, frog cleavage
Fig. 47.10, frog gastrulation
Fig. 47.14, three embryonic germ layers in vertebrates
Fig. 47.12, neurulation and frog organogenesis
Fig. 47.17, cellular shape and morphogenesis
Fig. 47.19, Cadherin
Fig. 47.12, neurulation and frog organogenesis
Fig. 47.21, frog fate map
Fig. 21.5, (Alberts etal, 2002) frogcellfate.jpg
Fig. 47.23, cytoplasmic determinants in frog
Fig. 47.24, newt "organizer" region
movie, 21_1.mov (MBOTC)
Fig. 47.12, (Campbell and Reece, 2002) chick blastula/gastrulation
Fig. 47.17, (Campbell and Reece, 2005) chick extraembryonic membranes
Fig. 40.17 (Purves et al., 1998) chick_membrane_development.jpg
Fig. 47.25, vertebrate limb development
Fig. 21.13, (Alberts etal, 2002) chicklimb.jpg
Fig. 47.26, Pattern formation, ZPA
Fig. 47.16, human embryogenesis
Fig. 55.12 (Raven and Johnson, 1995) mammalian_gastrulation.jpg
Fig. 3.6 (Marieb and Mallatt, 1997) human_blastopore
Fig. 3.11, (Marieb and Mallatt, 1997) Human_4week.jpg
Fig. 40.18, (Purves et al., 1998) human_40days.jpg
Fig 5_6 Patterns of Chordate Cleavage (Hidebrand and Goslow, 2001)

If time:
    (movie 21_4, from MBOTC)

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Hildebrand M., G. Goslow- 2001-Early Development. Chapter 5, Fig. 5.6. In <u>Analysis of Vertebrate Structure</u>. 5th edition. John Wiley & Sons, Inc. N.Y., N.Y.

Marieb E.N., J. Mallatt- 1997-<u>Human Anatomy</u>. 2nd edition. Figures 3.6, 3.11. Benjamin Cummings Press. Menlo Park, CA.

Purves W.K., G.H. Orians, H.C. Heller, D. Sadava- 1998-<u>Life: The study of biology</u>. Fifth edition. Figures 40.17, 40.18. W.H. Freeman and Company. Salt Lake City, Utah.

Raven P.H., G.B. Johnson- 1995-<u>Biology</u>. 3rd edition. Figure 55.12. Wm. C. Brown Publishers. Dubuque, Iowa.

Related issues:

In placental animals the fetus and the mother can swap not just molecules but also cells! This produces **microchimerism**. This can also happen through blood transfusions that carry stem cells from the donor to the recipient.

Choi C.Q- 2005-Baby to brain. Therapy clues from fetal cells that enter Mom's brain- Scientific American 293: (#5, Nov.) 22-23

Nelson J.L- 2008-Your cells are my cells- Scientific American 298: (#2, Feb) 72-79

Soares C- 2007-All in the family- Scientific American 297: (#1, July) 30

Vogel G- 2005-Controversial study finds an unexpected source of oocytes- Science 309: (7/29) 678-679

Other examples of **organizer regions** exist that create gradients of morphogens to guide animal development. Including in the development of part of the cerebral cortex, hair follicles, and the wings of insects.

Bénazet J-D., M. Bischofberger, E. Thecke, A. Goncalves, J.F. Martin, A. Zuniga, F. Naef, R. Zeller- 2009-A self-regulatory system of interlinked signaling feedback loops controls mouse limb patterning- Science 323: (#5917, 2/20) 1050-1053

Kicheva A., P. Pantazis, T. Bellenbach, Y. Kalaidzidis, T. Bittig, F. Jülicher, M. González-Gaitán- 2007-Kinetics of morphogen gradient formation- Science 315: (1/26) 521-525

Lewis J- 2008-From signals to patterns: Space, time, and mathematics in developmental biology- Science 322: (#5900, 10/17) 399-403

Maini P.K., R.E. Baker, C-M. Chuong- 2006-The Turing model comes of molecular age- Science 314: (12/1) 1397-1398

Mangale V.S., K.E. Hirokawa, P.R.V. Satyaki, N. Gokulchandran, S. Chikbire, L. Subramanian, A.S. Shetty, B. Martynoga, J. Paul, M.V. Mai, Y. Li, L.A. Flanagan, S. Tole, E.S. Monuki- 2008-Lhx2 selector activity specifies cortical identity and suppresses hippocampal organizer fate- Science 319: (#5861, 1/18) 304-309

Sick S., S. Reinker, J. Timmer, T. Schlake- 2006-WNT and DKK determine hair follicle spacing through a reaction-diffusion mechanism- Science 314: (12/1) 1447-1450

Zhang Z., Y. Lan, Y. Chai, R. Jiang- 2009-Antagonistic actions of Msx1 and Osr2 pattern mammalian teeth into a single row- Science 323: (#5918, 2/27) 1232-1234

Here is an article that describes some of the molecular details of **segmentation formation** in vertebrates..

Riedel-Kruse I.H., C. Müller, A.C. Oates- 2007-Synchrony dynamics during initiation, failure, and rescue of the segmentation clock- Science 317: (#5846, 9/28) 1911-1915

The study of **early embryo stages** of development are ongoing; including the formation of the zygote, the blastula, and other stages. Also the use of stem cells to study these stages. Here are some reviews and research articles that deal with embryology.

Barnes R.D- 1987-Invertebrate Zoology. 5th edition. Pgs. 86-89. Saunders College Publishing. Philadelphia, PA.

Behringer R.R- 2007-Dance of the embryo- Science 316: (5/4) 697-698

Brusca G.J., R.C. Brusca, S.F. Gilbert- 1997-Characteristics of metazoan development- Chapter 1, pgs 3-19, in Embryology: Constructing the organism. S.F. Gilbert and A.M. Raunio editors. Sinauer Assoc., Sunderland, MA.

Cruz Y.P- 1997-Mammals- Pgs. 459-489 Chapter 22 from Embryology: Constructing the organism- S.F. Gilbert and A.M. Raunio editors. Sinauer Assoc., Sunderland, M.A.

Elinson R- 1997-Amphibians- Chapter 20, pgs 409-436, in Embryology: Constructing the organism. S.F. Gilbert and A.M. Raunio editors. Sinauer Assoc., Sunderland, MA.

Fell P.E- 1997-The concept of larvae. Chapter 2, pgs 21-28, in Embryology: Constructing the organism. S.F. Gilbert and A.M. Raunio editors. Sinauer Assoc. Sunderland, MA.

Keller P.J., A.D Schmidt, J. Wittbrodt, E.H.K. Steizer- 2008-Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy- Science 322: (#5904, 11/14) 1065-1069

Kim K., P. Lerou, A. Yabuuchi, C. Lengerke, K. Ng, J. West, A. Kirby, M.J. Daly, G.Q. Daley- 2007-Histocompatible embryonic stem cells by parthenogenesis- Science 315: (1/26) 482-486

Kölsch V., T. Seher, G.J. Fernandez-Ballester, L. Secrano, M. Leptin- 2007-Control of *Drosophila* gastrulation by apical localization of adherens junctions and RhoGEF2- Science 315: (1/19) 384-386

Kurotaki Y., K. Hatta, K. Nakao, Y-I. Nabeshima, T. Fujimori- 2007-Blastocyst axis is specified independently of early cell lineage but aligns with the ZP shape- Science 316: (5/4) 719-723

McMahon A., W. Supatto, S.E. Fraser, A. Stathopoulos- 2008-Dynamic analysis of Drosophila gastrulation provide insights into collective cell migration- Science 322: (#5907, 12/5) 1546-1550

Marieb E.N., J. Mallatt- 1997-Basic embryology- Chapter 3, pgs. 48-64, in- Human Anatomy, 2nd ed. Benjamin/Cummings Press. Menlo Park, CA.

Nishida H- 1991-Induction of brain and sensory pigment cells in the ascidian embryo analyzed by experiments with isolated blastomeres- Development 112: (#2) 389-395

Schoenwolf G.C- 1997-Reptiles and birds- Chapter 21, pgs 437-458, in Embryology: Constructing the organism. S.F. Gilbert and A.M. Raunio editors. Sinauer Assoc., Sunderland, MA.

Stitzel M.L., G. Seydoux- 2007-Regulation of the oocyte-to-zygote transition- Science 316: (4/20) 407-408

Vogel G- 2005-Embryologists polarized over early cell fate determination- Science 308: (5/6) 782-783

This review examines how **cell-to-cell adhesion** molecules use tension dependent systems to help organize tissues in animals and considers similar issues in other forms of life.

Engler A.J., P.O. Humbert, B. Wehrle-Haller, V.M. Weaver- 2009-Multiscale modeling of form and function- Science 324: (#5924, 4/10) 208-212

The later stages of animal development involve organization of **organs**. There are some themes that are repeated in each organ's development.

Lu P., Z. Werb- 2008-Patterning mechanisms of branched organs- Science 322: (#5907, 12/5) 1506-1509

Slack J.M.W- 2008-Origin of stem cells in organogenesis- Science 322: (#5907, 12/5) 1498-1501

BIO 108   2010
Day 8, Lecture 21, Title: Animal Development II.
Text Readings: Campbell et al. (2008), pgs. 223-225, 366-373, 412-416, 442-447, 757-761.

Topics to cover:

> **Totipotency and Cloning**
> **Differentiation versus Determination**
> **Patterns of Gene Expression**
> **Fruit Fly Model**
> > **Maternal Cytosolic Factors**
> > **Induction of Segmentation**
> **Nematode Model**
> > **Vulva Induction**
> > **Apoptosis**
> **Homeotic Genes**

Have considered broad changes in shape during development (morphological changes),
        now will consider some aspects at the molecular level.
Will see that study of development in one animal can inform our understanding of other
        animals' development. Will also consider some aspect of development in plants.
This hopefully will illustrate how control patterns of gene expression seem to be highly conserved
        across eukaryotes...

Totipotency and Cloning
Totipotent cell, can make any other type of cell in organism
        many stem cells are totipotent, if limited to a few types of cells is called pluripotent.
        Fig. 47.22, cell lineages, *C. elegans*
Nematode, 959 mature cells, so have traced entire development sequence from zygote
        so zygote is totipotent
        How do these cells differentiate? Consider just two options...
        Do these cell differentiate by losing DNA? If so, it is a permanent change...?
        Or is it accumulation of cytosolic factors that matter? Non-permanent change?
To address this need to know if cloning is possible. If so, cells have the necessary items.
        Fig. 20.16, carrot cloning
                1950s, F.C. Steward at Cornell University
                many plant cells are totipotent
                Some plant cells are not totipotent, i.e. some phloem and xylem cells...
                SAM and RAM cells (shoot and root apical meristems)
                        are almost always embryonic and totipotent

Fig. 20.17, frog nuclear transplantation
       1950s, Briggs and King, and later work by Gurdon etal.
       used nuclei from mature frog cells
       put into enucleated egg or zygote cells
       Use various sources of cells. Do they develop normally? Sometimes...
       about 2% work if from mature endothelial cells (consider meaning of this)
             higher percentage if taken from blastula cells, so is something changed?
       ?? Is this because some cells lost totipotency, or induction of it was not done well?
Fig. 21.7, (Campbell and Reece, 2005) Dolly the sheep
       This demonstrated totipotency in mammals
       Papers by Wilmut (1998) and Wilmut et al. (1997)
       With cloning have ability to genetically alter one cell, and grow up adult
       Fig. 20.18, cloning mammals
       Needed to return nuclear material to zygotic state from differentiated state
             put cell through stress before did nuclear transfer
       But out of hundreds of attempts, got one success
             again, does this mean only a few cells retain totipotency??
       Turns out the DNA is not lost, but may be methylated, condensed, and so
             hard to return to the embryonic state.
       "Dolly" died young, had short telomeres and epigenetic issues
       So in theory can grow up entire tissue, organ, organism. Difficult in practice
             could put human gene put into cells
             with targeting for protein secretion
                  expression in mammary gland
                  so product is in milk
                  can be isolated from milk
                  in one case a protein to treat hemophilia
           so ability to clone is important for many biotechnological uses

Differentiation versus Determination
    Recall
       Determination: cell fate is set, but not yet achieved
       Differentiation: the process of achieving that final cell fate.
       both are aspects of development
    Fig. 18.16, muscle cell differentiation
    myoD gene   called a "master control" gene for muscle determination
            codes for transcription factor (TF)
            recall that dozens of TFs assemble for gene expression initiation complex
       alters expression of another gene, makes other TFs
       new combinations lead to expression of genes for muscle cell differentiation
       concept of master switch genes. myoD is one example

Internal/external factors that can influence such determination events.

Fig. 18.15, developmental information sources

TFs may be made in cell or may be imported

maternal growth factors donated during egg development

may be receptors for these factors in the cytosol?

(recall cytosolic determinants we considered in last lecture)

morphogins secreted from organizing regions (recall ZPA...)

implies existence of surface receptors?

(will later consider example of vulva induction in nematodes...)

Patterns of Gene Expression

Often see some degree of conservation of patterns of gene expression amongst animals...

even across different kingdoms often see similar systems of control

Fig. 21.17, homologous genes and pattern formation

note: similar patterns of genes used

patterns of expression for genes in fruit fly to mouse

implications:

studying flies can tell you something about mice

this is due to evolutionary heritage of homologous genes

duplication occurred with tetrapod lineage...

so animal model systems can inform human biology

go further and can see similarities from protists to plants...

Several model systems will now be considered that allow such comparisons...

Fruit Fly Model

Fig. 18.17, fruit fly development

Review aspects of fruit fly life stages...

Recall in animals, interrupted meiosis,

so secondary oocyte does little or no gene expression

So secondary oocyte (that will become the egg) gets maternal factors

(RNAs and proteins) from nurse cells of the mother

Consider then, maternal genetics influences offspring development?

early stage is coenocytic, mitosis without cytokinesis

later becomes cellular as does cytokinesis

later becomes segmented

larva, pupa, adult fly stages

Maternal Cytosolic Factors
Maternal factors are coded for by maternal DNA, and sent to next generation
Fig. 18.19 fruit fly, bicoid influences
      Maternal factors, maternal genotype matters a generation later...
      gene for this protein is in mom, she sends her mRNA
            unevenly distributed, more at anterior
            so bicoid protein made more at anterior
      establishes gradient of bicoid protein, when cellular stage is reached
            cells at anterior will have higher concentraiton of bicoid
            this will influence their development...
      So how is mRNA all at one end?
      other mRNAs (Nanos) accumulates at posterior end, so distribution in egg matters
Consider what would happen if zygote gets two mutated alleles of bicoid gene from
      heterozygous parents?   (Nothing, the problem would be in the next generation?)

Induction of Segmentation
Fig. 21.14, (Campbell and Reece, 2005) fruit fly, segmentation gene expression
      maternal effect gene bicoid tells embyro its anterior/posterior axis
      then get induction of segments and specialization of segments
Next see expression in embryo using its genotype
Fig. 21_UN425, (Campbell and Reece, 2005) Hierachy of Gene action
      gap genes
      pair rule genes
      segment polarity genes
            the above three will set segments,
            later ones help influence what in segment
      homeotic genes.... (recall that HOX genes are one type of homeotic gene)
      Notice, these are intracellular signals, starting with maternal factors, in the embryo.
So can follow pattern of gene expression once established then compare to other animals
      Once know the proper pattern, can try to account for errors that occur during it
            this has given us a system to study some birth defects...

Nematode Model
      Vulva Induction
(See handout, this example is not in the text...)
Fig. 21.16, (Campbell and Reece, 2005) cell signaling and induction
Can have extracellular signals that are held on surface, acting locally to neighbors
            or that are secreted, can act further away
See both of these in vulva induction in nematodes

Fig. 21.17, (Campbell and Reece, 2002) nematode vulva formation
Anchor cell secretes signal, acts at a distance, depending on its concentration
      Some epidermal cells induced to become vulval precursor cells

These are induced to express surface protein, an example of induction...

     can be bound by neighboring cell's receptors, so a local signal

Cell that gets lots of neighboring signals will undergo apoptosis, to form pore of vulva

  (Nematodes who do not form a pore end up with kids eatting their way out!)

so signals, signal transduction, gene expression all tied together

  note need for receptors of each signal type...

Apoptosis

Also called programmed cell death, a highly differentiated cell state (i.e. death...)

Note: some xylem cells do this to form tracheids and vessel members in plants...

  some bacteria do this when population is under stress...

  so this is not just something done in animals.

Example in nematode development

  Fig. 21.18a, (Campbell and Reece, 2005) apoptosis

  131 cells in *C. elegans* die as part of differentiation during normal development

  this also happens in humans, plants, etc... How to control cell death?

Example in mouse paw development.

  Fig. 11.21, mouse paw

  apoptosis is used here to remove the "webbing" from between the digits

Mechanism used.

  Fig. 11.20, apoptosis

  Death signal, bound by Death signal receptor

    Ced-9, inhibits signal cascade

  When Ced-9 is inactivated, cascade is turned on, this leads to cell death

    inactivation due to death signal receptor receiving death signal...

  This system is expressed and deployed in most

    animal cells, as a protection mechanism

humans use this as a means to kill pre-cancerous cells

    so to be a cancer cell need to avoid apoptosis mechanisms...

    apoptosis is a feature of our innate immune system

    some plants use it to inhibit virus spread in their bodies

Homeotic Genes

These are genes involved in influencing patterns of organ/limb development

In plants, use ABC gene system to determine flower parts for a species.

  Fig. 35.34, patterns of flower development

    Organ-identity genes, flower organs are sepals, petals, stamen, carpels...

    combination of signals lead to different organs in flowers

  Fig. 21.20b, (Campbell and Reece, 2002) organ identity tests in flowers

  Fig. 21.20c, (Campbell and Reece, 2002) organ identity tests in flowers

    altering expression of genes alters flower morph.

Animal homeotic genes, one major class are the HOX genes
 Fig. 18.18, fruit fly homeotic gene effects
 Often homeotic genes code for DNA binding proteins
  some have a homeodomain, 60 amino acid region
   may bind to DNA, may act as transcription factor in some cases
  so have 180 BP homeobox in DNA coding for above
 Thus homeotic genes have homeobox regions that code for homeodomains in protein...
  gives you a starting point to make a probe?
  however, not all genes with homeobox are homeotic genes
 Changes in expression of Hox genes alters body segment use
   Fig. 21.18, Hox gene expression
   Fig. 21.17, homologous genes and pattern formation

Use the model systems in nematodes, frogs, chickens, fruit flies and study patterns of gene expression through development. Then use this information to inform human studies.

Some common features of eukaryotic multicellular development:

 Source of information to guide development.
  Intracellular versus extracellular.
  This requires signals, receptors, transduction systems, responses.... etc.

 Patterns of gene expression,
  Begins with single gene expression changes, to whole sets
  Guided by transcription factor effects.
  Often this leads to organ/tissue/cellular specialization.
   Consider the examples of Hox genes, and ABC system.

Same patterns are used, and reused in different settings.
 Conservation of parts, allows for new patterns of development

Objectives:

Is all development influenced by the products of the genes within a single cell, or do gene products from some cells ever influence the determination and differentiation of other cells? What are examples of intracellular factors and extracellular factors that influence cell fate? Was it intracellular or extracellular factors that made cloning of animals so difficult to achieve? You should be able to describe examples of the role of such factors in differentiation, such as the role of bicoid in fruit flies, and how vulva induction is achieved in nematodes. Consider what role apoptosis can play during the development of an organism.

What are maternal effect genes, in which generation do they have effects, and whose genotype is having the influence on development in this case? Be able to describe the bicoid system as an example of a maternal effect system.

Be able to describe generally what happens to alter gene expression during cellular determination and differentiation. What is totipotency, and what would a cell that has this trait be able to do? Describe examples of experimental evidence of totipotency in adult animal and plant cells.

Controlling patterns of gene expression can involve information transduction systems. Can you give examples of several developmental situations in which there are signals passed between cells, suggest some hypothetical transduction systems that might be involved, and describe the likely results they would have?

Homeotic genes are found in animals, plants, and other eukaryotes. What are homeotic genes, and what might they control? Across animal species are the homeotic genes similar or different, and how would you account for this? What are homeoboxes and homeodomains, and in what types of molecules are they found?

Describe what changes you would look for, and in what types of molecules you would look, if you wished to study first the determination and then the differentiation of an adult cell type from an embryonic stem cell.

For review, see self-quiz questions #5, 6, and 7 of chapter 18. Self-quiz questions #6 and 8 of chapter 21. And self-quiz question #10 of chapter 35.

Needed overheads and items:

Fig. 47.22, cell lineages, *C. elegans*
Fig. 20.16, carrot cloning
Fig. 20.17, frog nuclear transplantation
Fig. 21.7, (Campbell and Reece, 2005) cloning, Dolly the sheep
Fig. 20.18, cloning mammals
Fig. 18.16, muscle cell differentiation
Fig. 18.15, developmental information sources
Fig. 21.17, homologous genes and pattern formation
Fig. 18.17, fruit fly development
Fig. 18.19, fruit fly, bicoid influences
Fig. 21.14, (Campbell and Reece, 2005) fruit fly, segmentation gene expression
Fig. 21_UN425, (Campbell and Reece, 2005) Hierachy of gene action
Fig. 21.16, (Campbell and Reece, 2005) cell signaling and induction
Fig. 21.17, (Campbell and Reece, 2002) nematode vulva formation
Fig. 21.18a, (Campbell and Reece, 2005) apoptosis
Fig. 11.21, mouse paw development and apoptosis
Fig. 11.20, apoptosis
Fig. 35.34, patterns of flower development
Fig. 21.20b, (Campbell and Reece, 2002) organ identity tests in flowers
Fig. 21.20c, (Campbell and Reece, 2002) organ identity tests in flowers
Fig. 18.18, fruit fly homeotic gene effects
Fig. 21.18, Hox expression
Fig. 21.17, homologous genes and pattern formation

Handout:
(Handout - Lecture 21 - Animal Development II.stm)
Fig. 21.16, (Campbell and Reece, 2005) cell signaling and induction
Fig. 21.17, (Campbell and Reece, 2002) nematode vulva formation

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-<u>Biology</u>. Eighth edition. Pgs. 223-225, 366-373, 412-416, 442-447, 757-761. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition. Figures 21.7, 21.14, 21.16, 21.18a, 21.UN425. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figures 21.17, 21.20a, 21.20b. Benjamin Cummings Press. San Francisco, CA.

Wilmut I- 1998-Cloning for medicine- Scientific American 279: (#6) 58-63

Wilmut I., A.E. Schnieke, J. McWhir, A.J. Kind, K.H.S. Campbell- 1997-Viable offspring derived from fetal and adult mammalian cells- Nature 385: (Feb. 27) 810-813

Related issues:

Other changes in **gene expression** during development have been documented in various species. Here are a few examples.

Boettiger A.N., M. Levine- 2009-Synchronous and stochastic patterns of gene activation in the *Drosophila* embryo- Science 325: (#5939, 7/24) 471-473

Brent A.E., G. Yucel, S. Small, C. Desplan- 2007-Permissive and instructive anterior patterning rely on mRNA localization in the wasp embryo- Science 315: (3/30) 1841-1843

Dequéant M-L., E. Glynn, K. Gaudenz, M. Wahl, J. Chen, A. Mushegian, O. Pourquié- 2006-A complex oscillating network of signaling genes underlies the mouse segmentation clock- Science 314: (12/8) 1595-1598

Ehebauer M., P. Hayward, A.M. Arias- 2006-Notch, a universal arbiter of cell fate decisions- Science 314: (12/1) 1414-1415

Lunyuk V.V., G.G. Prefontaine, E. Núñez, T. Cramer, B-G. Ju, J.A. Ohgi, K. Hutt, R. Roy, A. García-Díaz, X. Zhu, Y. Yung, L. Montoliu, C.K. Glass, M.G. Rosenfeld- 2007-Developmentally regulated activation of a SINE B2 repeat as a domain boundary in organogenesis- Science 317: (#5835, 7/13) 248-251

Olson E.N- 2006-Gene regulatory networks in the evolution and development of the heart- Science 313: (9/29) 1922-1927

Smith J., C. Theodoris, E.H. Davidson- 2007-A gene regulatory network subcircuit drives a dynamic pattern of gene expression- Science 318: (#5851, 11/2) 794-797

One specific set of genes that play a major role in animal development are the **HOX genes**.

Lemons D., W. McGinnis- 2006-Genomic evolution of Hox gene clusters- Science 313: (9/29) 1918-1922

Many **maternal factors**, such as proteins and RNAs have been found to be passed to the developing oocyte.

Brennecke J., C.D. Malone, A.A. Aravin, R. Sachidanandam, A. Stark, G.J. Hannon- 2008-An epigenetic role for maternally inherited piRNAs in transposon silencing- Science 322: (#5906, 11/28) 1387-1392

Schier A.F- 2007-The maternal-zygote transition: Death and birth of RNAs- Science 316: (4/20) 406-407

In addition to genetic influences there are **environmental influences** that occur in placental animals that alter fetal development.

Patterson P.H- 2007-Maternal effects on schizophrenia risk- Science 318: (#5850, 10/26) 576-577

Stix G- 2007-Selfless giving. Mom's brain chemical affects embryonic development- Scientific American 296: (#4, April) 20-22

Many **molecular systems** have been found to be involved in **pattern formation** during animal development, and the same systems are often used over and over again at different stages. Here are a some references that deal with a few such systems.

Bilić J., Y-L. Huang, G. Davidson, T. Zimmermann, C-M. Cruciat, M. Bienz, C. Niehrs- 2007-Wnt induces LRP6 signalosomes and promotes dishevelled-dependent LRP6 phosphorylation- Science 316: (6/15) 1619-1622

Fujisawa K., J.L. Wrana, J.G. Culotti- 2007-The slit receptor EVA-1 coactivates a SAX-3/Robo-mediated guidance signal in *C. elegans*- Science 317: (#5846, 9/28) 1934-1938

Hynes R.O- 2009-The extracellular matrix: Not just pretty fibrils- Science 326: (#5957, 11/27) 1216-1219

Jacob L., L. Lum- 2007-Deconstructing the hedgehog pathway in development and disease- Science 318: (#5847, 10/5) 66-68

Sanes D.H., T.A. Reh, W.A. Harris- 2006-Polarity and segmentation- Chap 2, pgs 29-55, in Development of the Nervous System, 2nd edition. Elsevier Academic Press, N.Y., N.Y.

Strome S., R. Lehmann- 2007-Germ versus soma decisions: Lessions from flies and worms- Science 316: (4/20) 392-393

Tabin C.J., A.P. McHahon- 2008-Grasping limb patterning- Science 321: (#5887, 7/18) 350-352

One trap we often fall into is thinking that if we just can know all the genetic factors involved we can determine the phenotype. This is the (often false) premise taken by many genomic studies. This article points out the **limits of** our ability to predict phenotype from **genomic data**.

Demitzakis E.T., A.G. Clark- 2009-Life after GWA studies- Science 326: (#5959, 10/9) 239-240

There are **homeotic genes** in other species. Here are some reports of the use of homeotic gene systems in the control of development of **plants**.

Benfey P.N., D. Weigel- 2001-Transcriptional networks controlling plant development- Plant Physiology 125: 109-111

Hartwell L., L. Hood, M.L. Goldberg, A.E. Reynolds, L.M. Silver, R.C. Veres- 2000- *Arabidopsis thaliana*: A genetic portrait of a model plant- Chapter 19, pgs. 655-680, in, Genetics: From Genes to Genomes- McGraw Hill. Boston, MA.

Sharma V.K., J.C. Fletcher- 2002-Maintenance of shoot and floral meristem cell proliferation and fate- Plant Physiology 129: 31-39

The changes in genetic **expression patterns** and **combinations of proteins** that occur during the process of **determination** are slowly being nailed down.

Bar-Yam Y., D. Harmon, B. deBivort- 2009-Attractors and democratic dynamics- Science 323: (#5917, 2/20) 1016-1017

Costanzo M., A. Baryshnikova, J. Ballay, Y. Kim, E.D. Spear, C.S. Sevier, H. Ding, J.L.Y. Koh, K. Toufighi, S. Mostafavi, J. Prinz, R.P. St.Onge, B. Van der Sluis, T. Makhnevych, F.J. Vizeacoumar, S. Alizadeh, S. Bahr, R.L. Brost, Y. Chen, M. Cokol, R. Deshpande, Z. Li, Z-Y. Lin, W. Liang, M. Marback, J. Paw, B-J. San Luis, E. Shuteriqi, A.H.Y. Tong, N. van Dyk, I.M. Wallace, J.A. Whitney, M.T. Weirauch, G. Zhong, H. Zhu, W.A. Houry, M. Brudno, S. Ragibizadeh, B. Papp, H. Bussey, G.D. Bader, A-C. Gingras, Q.D. Morris, R.M. Kim, C.A. Daiser, C.L. Myers, B.J. Andrews, C. Boone- 2010-The genetic landscape of a cell- Science 322: (#5964, 1/22) 425-431

For a nice review of some work on cloning and the production of **stem cells** and their uses to make new cells for tissure repair see:

Gurdon J.B., D.A. Melton- 2008-Nuclear reprogramming in cells- Science 322: (#5909, 12/19) 1811-1815

Minkel J.R- 2008-Potent alternative. Reversed-engineered human stem cells may leap frog the embryonic kind- Scientific American 298: (#2, Feb) 16-17

Stem cells tend to be found in locations called **stem cell niches**. In these locations extracellular signals help to maintain the different populations of stem cells seen in animals.

Discher D.E., D.J. Mooney, P.W. Zandstra- 2009-Growth factors, matrices, and forces combine and control stem cells- Science 324: (#5935, 6/26) 1673-1677

Li L., H. Clevers- 2010-Coexistence of quiescent and active adult stem cells in mammals- Science 327: (#5965, 1/29) 542-545

Mathur D., A. Bost, I. Driver, B. Ohistein- 2010-A transient niche regulates the specification of *Drosophila* intestinal stem cells- Science 327: (#5962, 1/8) 210-213

Apoptosis is one possible result of differentiation. It can play an important role in animal development.

Davidson L.A- 2008-Apoptosis turbocharges epithelial morphogenesis- Science 321; (#5896, 9/19) 1641-1642

Toyama Y., X.G. Peralta, A.R. Wells, D.P. Kiehart, G.S. Edwards- 2008-Apoptotic force and tissue dynamics during *Drosophila* embryogenesis- Science 321: (#5896, 9/19) 1683-1686

BIO 108   2010
Day 9, Lecture 22, Title: Phylogeny and Systematics.
Text Readings: Campbell et al. (2008), chapter 26.

Topics to cover:

      Classical classification
      Post-evolutionary theory Classification
            Numerical taxonomy
            Cladistics
      Confounding issues
            convergent evolution
            divergent evolution
            horizontal gene flow, endosymbiosis
      DNA data and Molecular data
            molecular clocks
      Summary

Classical classification
50 years ago life was simpler...
      Just three kingdoms of life: Plants, animals and bacteria (Monera).
The continuing revision of systematics I see as a measure of how immense our ignorance of life
      continues to be, even to this day.
One shift has been the addition of a new level of classification
         Fig. 26.3, hierarchical classification
      KPCOFGS → DKPCOFGS
      "King Philip Cried Oh For Goodness Sake!" becomes
      "Dear King Philip Cried Oh For Goodness Sake!"   or similar phrase...
         Fig. 26.3, hierarchical classification
Started with Linnaeus, by his time were enough described species that needed organization
      His was not the only classification scheme, dozens of others. Why did he prevail?
      Naming of type specimens for person who donated it to him.
      Ease of use.
         Binomial, morphological, but no assumption of a common ancestor...
      Hit on a set of traits to use that were fairly robust. using sexual parts.
         these are subjected to natural selective pressures, it has mostly held up over time
         an artificial classification that happens to fit phylogenies fairly well...
      pg. 113 (Schiebinger, 1996) LovesOfPlants.jpg
         An excellant article! Note scandelous nature of this scheme.
Pre-evolutionary theory
      Classification was bookkeeping, as was Linnaeus' work
      No attempt to figure out an actual historical sequence of evolutionary changes

Post-evolutionary theory Classification:
>   Was a tool to organize specimens.
>   Now wish to get sequence of changes (evolutionary lineage) and relationships..

Issues that confound these attempts
>   Convergent evolution: leads to analogous traits
>>   these have similar functions, but come from distinct lineages
>>>   natural selection for similar function over time leads to convergence
>>   examples: shapes of swimming fish, mammals, birds
>>>   wing shape of birds, bats, bees
>   Divergent evolution: leads to homologous traits
>>   common ancestral state subjected to different natural selective pressures
>>>   so different species modify anceatral structure
>>>   results in distinct functions all modified from common past structure
>>   eamples: Forelimbs of animals become wings, limbs, arms, fins...
>>>   leaves of plants become petals, carpels, fruit...

How to put together a proposal of phylogeny?
>   In the past, after life long study, a proposal was published
>>   each person chooses traits for use in their classification schemes
>>   Argument over the resulting classification system then ensues.
>>   This was highly subjective, not easily checked by people entering the field
>   **Numerical taxonomy**, was popular in past, and used again today for molecular data...
>   This approach became popular after computer use became widespread
>   Use of all character sets possible: 100-1000s in some cases
>   Idea being that it is not possible to know which traits are analogous vs. homologous traits
>>   so use as many traits as possible to avoid confounding issue
>   Nice in its being objective, bad in that you have to measure all these traits for each species
>>   and many of these traits are considered not to be useful
>>   this does not focus attention on new or distinguishing character changes
>   Aspects of this approach remain in molecular and genomic data uses...
>>   Use computer software to determine relatedness,
>>>   but using different software often gives different answers!
>   **Cladistics**, very popular method used today
>   Approachable, and testable, but does require some assumptions
>   Assumes that can define in and out groups, so must know something already?
>>   in group: are known to be the descendants of a common ancestor
>>   out group: thought to be the immediate relative of the above, but not in it
>>   problem of convergence is still here within the in-group species?
>   Need to identify shared ancestral traits
>>   These are seen in out group, nearest relative who lacks certain ancestral traits
>   Need to identify derived traits shared by descendants as acquired
>>   Implies an in group, once a derived trait shows up descendants have it
>>   and within this in group can identify shared derived traits that distinguish taxa

Use this information to construct Cladogram and to identify Clades
        cladogram is a representation, a hypothesis of relationships
        A clade is also a hypothesis, a group of species sharing a common ancestor
            Note the use of out group to "anchor" the tree,
                i.e. defines the clade by contrast.
Good features of cladistics:
        forces identification of homologous (i.e. shared derived) traits
        Focuses on distinguishing traits
        also identifies shared ancestral traits, which are present but not distinguishing
        can be tested by looking at other traits in the species being compared
            for instance, produce a cladogram using morphological traits
                test it by looking at developmental traits, or molecular data...
        Once a cladogram is made, other groups can be added or attached to it...
Example of use for a simple (1/0) character sets
        Fig. 26.11, cladistics
            (Opps! Do all vertebrates have backbones, or just vertebrae?
                Remember the difference between Vertebrates and Osteichthyans?)
        ends up using just a few traits
        Need:
            to know traits in organisms
            to identify an outgroup, that lacks traits but is a close relative
        Note what happens if try to insert birds into this? (see handout)
            Bird: hair 0, egg 1, four walking legs 0, jaws 1, vertebrate 1
            note how this may place birds somewhat oddly in this cladogram
                bird1.tif
            putting birds with fish makes the amniotic egg analogous?
                bird3.tif
            Or can modify the traits used
                in this case birds end up in amniotes, and not fish...
            This system forces modification and clarification of choices
                that makes it very useful
        Fig. 25.12, (Campbell and Reece, 2002) cladogram of mammals
            note new ingroup, new outgroup, new characters,
            note mammals are a clade
principle of parsimony: in absence of other data or compelling reasons...
        select the model that requires the fewest assumptions
            meaning fewest gain or loss of character states
         finding new data can then alter the model
        reality is not required to be parsimonous, just most likely to be...

Confounding issues:
Revisit the problem of **convergent evolution**
        produces analogous traits that look similar but not shared by a common ancestor
    need to use homologies coming from shared ancestrial traits
        Ex: wings as a trait, would include bats, birds, bees?? (an analogy)
            (contrast to wings of birds and forelimbs of turtles, homologies)
        ex: four chambered hearts in birds and mammals?
                Fig. 25.16, (Campbell and Reece, 2005) analogous traits
            eyes in squid and eyes in mammals, etc...
        convergence implies similar natural selection?
            Fig. 26.7, convergent evolution
        burrowing animals need similar biomechanical features

Revisit problem with **divergent evolution**
        loss of traits can occur, so absence as well as presence can give information
            for instance, many parasites have lost many traits...
        some birds do not have functional wings, yet their ancestor did,
            if do not know ancestor where to place wingless birds?

So typically make use of multiple character sets, in a parallel manner as cross checks of each other
    These often include: classical phenotypic, embryonic, biochemical data
        also include data from molecular studies (i.e. gene sequences, genomes)
Attempting to produce monophyletic taxa.
        Fig. 26.10, monophyletic, paraphyletic, polyphyletic
    Monophyletic, common ancestor and all descendants included
        Animal Kingdom, Plant Kingdom, these can each be called a clade this way
        Fig. 26.4, Order Carnivora
            This shows an Order that is thought to be monophyletic
            Notice that not ALL carnivores are shown in this cladogram
                but if did include them all would fit in this order, so it works
        Fig. 26.10, monophyletic, paraphyletic, polyphyletic
    Polyphyletic, multiple ancestors
        Algae, Zooplankton, Herbivores...
        these are common terms and do not represent clades...
    Paraphyletic, common ancestor, but not all descendants included
        Reptiles, once thought to be a clade, now revised so no longer considered to be
            Fig. 25.18, (Campbell and Reece, 2002) paraphyletic example
                note reptiles include birds, lizards and snakes
        Old view of reptiles was paraphyletic, but birds now included so is monophyletic
            Fig. 34.24, amniotic phylogeny

Some other confounding possibilities
How to deal with **horizontal gene flow, endosymbiosis**?
Fig. 26.22, horizontal gene transfer
suddenly you find a gene from bacteria in eukaryotes?
consider what this does to attempts to reconstruct relationships...
What is horizontal gene transfer? vs. vertical gene transfer?
vertical gene flow: from one generation to another, down through lineage
horizontal gene flow: due to transformation, transduction, hybridization, etc...
can cross lineages and confound our attempts to reassemble relations
Fig. 28.7, (Campbell and Reece, 2002) lots of horizontal gene transfer
early in life there might have been lots of horizontal gene flow
this may limit our ability to identify the characters found in a common ancester?
may have been a common ancestral community rather than just one species?
This also has implications for how new species can arise...

DNA data and Molecular clocks
Use of DNA data to reconstruct phylogeny (This is the modern form of Numerical Taxonomy?)
Fig. 26.8, aligning DNA
have to know you are using similar regions of DNA, i.e. same gene...
have to identify insertion and deletion sections
convergence in genes? with four bases... can get CCC alot by chance?
need for large number of sites in analysis
many early studies used only dozens to hundred sites for only one gene
tendency now is to use many more sites and genes, even whole genomes..
Changes accumulate in various parts of DNA at various rates
not all DNA mutates equally
How much a region of DNA changes depends in part on degree and steadiness of selection
Fig. 26.18, homologous genes
Want to use genes that are similar initially
Use of introns vs. exons, need to align and find variable regions...
Note that this is not cladistics...
since do not know ancestral trait, these are not anchored, no outgroup
Compare similar genetic sections have to choose section based on time scale
Use highly conserved regions for comparisons over long periods
ribosomal genes, transfer RNA genes
this works for hundreds of millions to billions of years
good for comparing domain or kingdom or phyla level of taxa...
Use less conserved regions for comparisons over shorter periods
use differences in introns of a gene, changes in transposon positions
good for comparison between species in a genus
or individuals in a species (i.e. DNA fingerprinting...)

Example from text
    Fig. 26.15, parsimony and molecular systematics
    For three species are three possible relationships
    Using molecular data, assuming they got the same gene regions correctly
        here uses just four nucleotide sites, in reality use 100s to 1000s.
    Consider shifts needed for just site #1
    How did they get the ancestral DNA state?
        Rarely get DNA from millions of years ago, so likely assumed?
        so do not always know ancestral states, and no outgroup
          so this is not a cladistical analysis
    See the figure in the text for the final best tree based on the data, a hypothesis...
Molecular clocks
    Uses of changes in nucleotide sequences to estimate time back to common ancestor
    May allow an estimate of time of divergence, but has assumptions
    For deep time estimates want items with continuous similar selection levels
        so rate of change in nucleotide sequence is consistent
          (for instance 3 changes in the section of DNA per million years...)
        (note: later will see that this is not consistent with punctuated evolution)
    Need for skepticism
        Fig. 26.20, HIV molecular clock
        If assume rate of evolution is constant, and assume knowledge of ancestral state
          then can project back to when divergence occurred
        Fig. 26.20, HIV molecular clock
          but note:
          38 points above line
          72 points below line
        what if rate of change, changes...?
        Many estimates from molecular clock data have over estimated date of divergence
          in some cases predicting divergence hundreds of millions of years
          beyond oldest discovered fossils of diverged groups!

Revisions have been made to phylogenies, in part based on molecular data
    Figs. 32.10 and 32.11, traditional vs. molecular comparison, invertebrate relationships
    All phyla shown here are in same order, but note the different relationships shown
    Acquisition vs. loss of traits
        body cavity acquired, and then lost
        Full digestive cavity, lost (in both cases see platyhelminthes, Planaria)
    New traits suggested for comparison
        extracellular matrix of
          nematodes and arthropods now considered important
          before pseudocoelomate state considered important
        and larval traits

Summary

Ultimate goal is to use molecular, phenotypic, fossil data and produce a hypothesis of phylogeny
        that is supported by the most data.
        Fig. 26.13, ultrametric trees
Fossil data can give dates, but fossil record is incomplete, so tends to underestimate ages?
Molecular data can also estimate dates of divergence, but tends to overestimate?
                note, it extends the date of divergence back...
The best work uses several data sets of distinctly different types, as cross checks on each other.
        But most studies use only molecular data, why is that?
                It is the current hot topic. Fairly easy to do with techniques that are available.

This allows us to estimate the relationships of all known life.
        Fig. 26.21, three domains of life

Importance of systematics and knowing species relationships is critical in biology
        we are losing the ability to identify species...
        there are still many unknown species
        these phylogenies are the source of new knowledge....

Objectives:

If given two simple phylogenetic trees you should be able to evaluate which is most parsimonious. How is parsimony used to choose the "best" phylogenetic tree? Be able to use a phylogenetic tree to evaluate the relatedness of the taxa shown in it.

There are some terms worth knowing: In group, out group, analogous, homologous, shared ancestral characters, shared derived characters, monophyletic, polyphyletic, paraphyletic, clades and cladograms. Be able to use each of these terms.

What must be known in order to carry out a cladistical analysis? What assumptions are made in the assembly of a cladogram? Given an appropriate data set, be able to construct and use a cladogram.

What are some benefits and pitfalls of using molecular data in classification? What does information from either molecular data or fossils sometimes allow to be added to a hypothetical phylogeny? What assumptions are made in the use of "molecular clocks," and what information can be obtained with their use? What makes a set of traits homologous versus analogous? Be able to identify a set of analogous traits produced by convergence, and a set of homologous traits produced by divergence.

What is the difference between vertical gene flow and horizontal gene flow? What is a process or event that is an example of each of these? What is the consequence of each of these on attempts to reconstruct evolutionary relationships?

For review, see self-quiz questions #1, 3-9 of chapter 26.

Needed overheads and items:

       Fig. 26.3, hierarchical classification
       Fig. 26.3, hierarchical classification
       pg. 113 (Schiebinger, 1996) LovesOfPlants.jpg
       Fig. 26.11, cladistics
       bird1.tif
       bird3.tif
       Fig. 25.12, (Campbell and Reece, 2002) Cladogram of mammals
       Fig. 25.16, (Campbell and Reece, 2005) analogous traits
       Fig. 26.7, convergent evolution
       Fig. 26.10, monophyletic, paraphyletic, polyphyletic
       Fig. 26.4, Carnivore order
       Fig. 26.10, monophyletic, paraphyletic, polyphyletic
       Fig. 25.18, (Campbell and Reece, 2002) paraphyletic example
       Fig. 34.24, amniote phylogeny
       Fig. 26.22, tree of life
       Fig. 25.7, (Campbell and Reece, 2002) lots of horizontal gene transfer
       Fig. 26.8, aligning DNA
       Fig. 26.18, homologous genes
       Fig. 26.15, parsimony
       Fig. 26.20, HIV molecular clock
       Fig. 26.20, HIV molecular clock
       Figs. 32.10 and 32.11, traditional vs. molecular comparison, inverts
       Fig. 26.13, ultrametic tree
       Fig. 26.21, three domains of life

Handout:

Handout - lecture 22.stm
       bird1.tif, bird3.tif

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Phylogeny and the tree of life. Chapter 26. Pages 536-555. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition. Fig. 25.16. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figures 25.7, 25.12, 25.18. Benjamin Cummings Press. San Francisco, CA.

Schiebinger L- 1996-The loves of the plants- Scientific American 274: (#2) 110-115

Related issues:

These studies look at aspects of **horizontal gene flow**. How it occurs, when is it common, and its implications for attempts to reconstruct phylogenies.

Keeling P.J- 2007-Deep questions in the tree of life- Science 317: (#5846, 9/28) 1875-876

Keeling P.J- 2004-Diversity and evolutionary history of plastids and their hosts- American Journal of Botany 91: (#10) 1481-1493

McInerney J.O., D. Pisani- 2007-Paradign for life?- Science 318: (#5855, 11/30) 1390-1391

The Nasonia Genome Working Group- 2010-Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species- Science 327: (#5963, 1/15) 343-348

Sorek R., Y. Zhu, C.J. Creevey, M.P. Francino, P. Bork, E.M. Rubin- 2007-Genome-wide experimental determination of barriers to horizontal gene transfer- Science 318: (#5855, 11/30) 1449-1452

Instead of using just data from gene sequences, now data from entire genomes (**genomic systematics**) are also being used to try to reconstruct phylogenies. As with all methods, this approach has its own problems and limitations.

Löytynoja A., N. Goldman- 2008-Phylogeny-aware gap placement prevents errors in sequence alignment and evolutionary analysis- Science 320: (#5883, 6/20) 1632-1635

Pennisi E- 2008-Building the tree of life, genome by genome- Science 320: (#5884, 6/27) 1716-1717

Rokas A- 2006-Genomics and the tree of life- Science 313: (9/29) 1897-1898

Wong K.M., M.A. Suchard, J.P. Huelsenbeck- 2008-Alignment uncertainty and genomic analysis- Science 319: (#5862, 1/25) 473-476

For more on creating **phylogenetic trees** and doing **cladistics** and other methods see:

Grant V- 2003-Incongruence between cladistic and taxonomic systems- American Journal of Botany 90: (#9) 1263-1270

Grant V- 1998-Primary classification and phylogeny of the Polemoniaceae, with comments on molecular cladistics- American Journal of Botany (#6) 741-752

Pearson P.N- 2003-Phylogenetic tree shape- Chap. 2.3.7, pgs. 192-195, in Palaeobiology II. D.E.G. Briggs and P.R. Crowther editors. Oxford, U.K.

Wilkinson M- 2003-Phylogenetic Analysis- Chap. 5.3.1, pgs. 509-515, in Palaeobiology II. D.E.G. Briggs and P.R. Crowther editors. Blackwell Pub. Oxford, U.K.

These articles notes the uses of, and some of the difficulties with, **molecular clocks**, and how they are often used to estimate the dates of divergences between two lineages.

Cooper A., N. Grassly, A. Rambaut- 2003-Using molecular data to estimate divergence times- Chap. 5.3.6, pgs. 532-534 in <u>Palaeobiology II</u>. D.E.G. Briggs and P.R. Crowther editors. Blackwell Pub. Oxford, U.K.

Sanderson M.J., J.L. Thorne, N. Wikström, K. Bremer- 2004- Molecular evidence on plant divergence times- American Journal of Botany 91: (10) 1656-1665

Most studies of **phylogeny** today involve the use of **molecular data**, but there are still complications. Often there are arguments over which regions of DNA to use; the coding or **non-coding regions**. Some argue that the **codon regions** can tell us about the **codon usage patterns** of various species. Then there are many ways to analyze the DNA sequence data from various species, and it has been found that different popular software systems give different **sequence alignments**, resulting in very different conclusions when applied to the same data set. Also various means of **gene duplications** and **deletions** can make the gene sequences of two species non-homologous (i.e. **paralogs**), complicating the use of molecular data still further.

Allman E.S., J.A. Rhodes- 2010-Trees, fast and accurate- Science 327: (#5971, 3/12) 1334-1335

Cavalier-Smith T- 1989-Archaebacteria and archezoa- Nature 339: (May 11) 100-101

Christianson M.L- 2005-Codon usage patterns distort phylogenies from or of DNA sequences- American Journal of Botany 92: (#8) 1221-1233

Pirie M.D., M.P.B. Vargas, M. Botermans, F.T. Bakker, L.W. Chatrou- 2007-Ancient paralogy in the cpDNA trnL-F region in Annonaceae: Implications for plant molecular systematics- American Journal of Botany 94: (#6) 1003-1016

Roch S- 2010-Toward extracting all phylogenetic information from matrices of evolutionary distances- Science 327: (#5971, 3/12) 1376-1379

Rokas A- 2008-Lining up to avoid bias- Science 319: (#5862, 1/25) 416-417

Shaw J., E.B. Lickey, E.E. Schilling, R.L. Small- 2007-Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III- American Journal of Botany 94: (#3) 275-288

Wong K.M., M.A. Suchard, J.P. Huelsenbeck- 2008-Alignment uncertainty and genomic analysis- Science 319: (#5862, 1/25) 473-476

Here are a few **examples** of attempts to reconstruct the **phylogeny** of different lineages. They deal with: humans, beetles, cats, primates, green algae, and flowering plants.

Crepet W.L., K.C. Nixon, M.A. Gandolfo- 2004-Fossil evidence and phylogeny: The age of major angiosperm clades based on mesofossil and macrofossil evidence from Cretaceous deposits- American Journal of Botany 91: (10) 1666-1682

Driscoll C.A., J. Clatton-Brock, A.C. Kitchener, S.J. O'Brien- 2009-The taming of the cat- Scientific American 300: (#6, June) 68-75

Harrison T- 2010-Apes among the tangled branches of human origins- Science 327: (#5965, 1/29) 532-534

Hunt T., J. Bergsten, Z. Levkanicova, A. Papadopoulou, O. St.John, R. Wild, P.M. Hammond, D. Ahrens, M. Balke, M.S. Caterino, J. Gómez-Zurita, I. Ribera, T.G. Barraclough, M. Bocakova, L. Bocak, A.P. Vogler- 2007-A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation- Science 318: (#5858, 12/21) 1913-1916

Lewis L.A., R.M. McCourt- 2004-Green algae and the origin of land plants- American Journal of Botany 91: (10) 1535-1556

Janećka J.E., W. Miller, T.H. Pringle, F. Wiens, A. Zitzmann, K.M. Helgen, M.S. Springer, W.J. Murphy- 2007-Molecular and genomic data identify the closest living relative of the primates- Science 318: (#5851, 11/2) 792-794

Wong K- 2009-The human pedigree- Scientific American 300: (#1, Jan) 60-63

BIO 108   2010
Day 9, Lecture 23, Title: Mutations.
Text Readings: Campbell et al. (2008), pages 297-302, 316-318, 344-346, 356-363, 373-377,
        432-442, 495-496, and 576-577.

Topics to cover:

What is a Mutation?
Small Scale Changes.
Duplications and Excisions.
Non-Gene Changes.
Chromosomal Changes.
Genomic Level Changes.
Cross Species Changes.
Summary.

What is a Mutation?
        Any heritable change in the genome.
        The advantage?
                Allows the production of new genetic material or new genetic combinations or
                        new control systems.
                This is the source of new variation from which to select, allowing evolutionary
                        changes...
                        Note: sexual life cycle makes new combinations of these new items.
        The costs? Most changes will break what is working. And so are deleterious.
Will cover some levels of changes that are possible
        1) Small scale changes. 2) Duplications/excissions. 3) Genetic vs. Non-genetic changes.
        4) Chromosomal changes. 5) Genomic duplication and redistribution.
        6) Cross-species changes.

Small Scale Changes.
        Fig. 17.23, types of point mutations
                Frameshift changes, can alter all amino acids coded for downstream of change
        Fig. 17.5, genetic code
                not all mutations equal, change in first position in a codon is worst
        Single nucleotide changes, called Point mutations
                Often result in single amino acid changes
                        Fig. 17.23, types of point mutations
                        silent mutations and genetic code
                        Mis-sense mutations
                                one amino acid change, may lead to altering of the phenotype

Non-sense mutations

     change one codon to a stop codon, so rest of protein not made

Note example in sickle cell anemia

Fig. 17.22, single nucleotide change, sickle cell anemia

     one glutamate changed to a valine

     valine is hydrophobic, promotes protein clumping in red blood cell

Above are all in exons, could have nucleotide changes in introns or other areas

Repair systems

     Fig. 16.18, DNA repair mechanisms

     1/10000 errors occur due to replication, repair systems convert this to 1/billion

          consider what this implies for number of cell divisions possible...

     repair systems very active in eukaryotes, with large genomes

          not very common in prokaryotes, with smaller genomes

     excision done by nucleases

     DNA polymerase to replicate from one strand

     DNA ligase to make phosphoester bonds

     Enzymes needed for repair systems are coded for by genes

          mutations in these genes can lead to major problems...

Duplications and Excisions.

Unequal crossing over

     Fig. 21.12, unequal crossing over

     could occur during mitosis (but rare), or more commonly during meiosis

     may produce duplications of genes in region affected

Transposons

     Simple transposons, account for about 40% of our genome

     Fig. 18.19a, (Campbell and Reece, 2005) transposon

     Fig. 18.17, (Campbell and Reece, 2002) transposon insertion and target sites

     Fig. 21.9a, transposon movement

          inverted repeat regions

          transposase: Bind DNA, cut and insert, like a restriction enzyme?

          note role of DNA polymerase and ligase, like a repair system?

          target site. What could this do to gene expression?

     Composite transposons

     Fig. 18.19b, (Campbell and Reece, 2005) composite transposon

          Two simple transposons about a gene inbetween them

          so can carry other genetic elements other than simple transposon ones...

     Replicative transposition (i.e. retrotransposons)

          Fig. 21.9b, retrotransposons

          mRNA of transposition converted to DNA, via reverse transcriptase

          then inserted in new location

          this allows genetic duplication

          can lead to multiple repeat regions

Could move just exons, and not entire genes?
>   Fig. 21.14, exon reshuffling
>   This allows duplication of just domains of polypeptides
>   ex: ATP binding site... can duplicate exon coding for it over and over
Can produce families of related genes
>   Fig. 21.13, gene family evolution
>   ex: globin gene family
>   pseudogenes, duplicated but lack promoters
>   many other gene families known

Non-Gene Changes.

Not all changes are in genes.

Many, such as transposons just described, can be in non-gene regions...
>   Recall that less than 2% of human DNA has protein-coding genes
>   most is non-protein gene regions... may code for RNAs instead!

Changes in introns
>   Fig. 18.8, eukaryotic gene and its control regions
>   if need to splice them out, what if alter sequence of intron and is not cut out?
>   >   mutation can alter splicing ability
>   recall that some introns spliced out form RNAs that act in spliceosomes
>   >   so mutation may alter splicing of other gene products?

Review distal control elements in eukaryotes and what could move
>   >   enhancers
>   >   gene away from proximal control elements
>   >   >   or from promoter, making pseudogenes
>   >   gene to/from suppressed regions
>   >   >   such as nearer to centromere

This can lead to release of a gene from control.
>   Fig. 18.20, genetic changes for oncogene production
>   Could convert a proto-oncogene to an oncogene?

"jumping genes" and Barbara McClintock

movement of genetic material altered other gene expression
>   Fig. 21.8, McClintock

So could have same genes, but alter control elements
>   and this could alter genetic expression without altering sequence in actual genes...

Chromosomal Changes.

        Fig. 15.15, alternations in chromosome structure

        Deletions, Insertions, Inversions, Duplications, Translocations

        Via large scale transposition, uneven crossing over, radiation damage, etc...

        This can move genes to new regions of chromosomes or to new chromosomes

        See changes like this between species. (Han et al. 2007)

                Pennisi etal 2007 - primate tree.jpg

                This allowed revision of primate phylogenetic tree.

Genomic Level Changes.

        There are other ways to alter chromosomes in addition to above...

        Meiotic non-disjunction

                Fig. 15.13, meiotic nondisjunction

                a failure to meet the demands of the principle of segregation

                produces extra chromosomal copies. Trisomy, etc.. Down's syndrome...

                may result in new chromosomes

        Autopolyploidy

                Fig. 24.10, autopolyploidy

                failure of segregation for entire genomic sets!

                alters ploidy in massive ways

                Entire extra set kept through meiosis.

Cross Species Changes.

        Concept of horizontal gene flow, across lineages vs. vertical gene flow down lineage

        Cytosolic genome

                Fig. 6.9 animal cell

                genes in mitochondria and chloroplasts there from other species...

                        genes moved form organelles to nucleus

                        process can go in the other direction

        Primary enodsymbiosis

                Fig. 25.9, endosymbiosis

                between prokaryote and eukaryote

                so moves genes between domains

        Secondary Endosymbiosis

                Fig. 28.5, (Campbell and Reece, 2002) secondary endosymbiosis

                moves entire genomes for recombination

                so entire genomes can move between eukaryotes

        Implication: horizontal gene flow, may confound attempts to determine lineages of life?

        Allopolyploidy

                Fig. 24.11, allopolyploidy

                genetic combination of two distinct species

                        creates a hybrid with new genetic info

                        very common in plants, seen in animals as well

        Transformation and transduction

                these can move genes between species

Transformation of cells lining our digestive tract
Viruses can insert genes, or alter our genes... So promote cancer.
     recall that we have many viral-derived DNA segments in our genome

Summary.
Small scale vs. large scale changes and selection
    leading to variation amongst individuals of a species
        Fig. 20.17, (Campbell and Reece, 2005) DNA fingerprinting
        Shows variation amongst humans in our DNA
        This can lead to speciation
        as in allopolyploidy
        selection for new combinations, creation of new allelic forms, new genes
    or leading to cancer
        if in somatic cells
        selection against somatic mutations
    Note existence of infectious cancer found in dogs, other found in Tasmanian devil
        (Zimmer, 2006; Holtcamp, 2007).
        When does it stop being defined as cancer, and start being a parasite?
        Wouldn't the ultimate goal for a cancer to be cross-species infectious?

Mutation and sexual life cycles allows new genetic combinations of material and formation of new genetic material. So it is essential for speciation.   Without the production of new variation there would be no new material for natural selection to work upon...

    Most mutations are deleterious, some few are advantageous.
        This produces variation...
    Need natural selection, differences in fitness, to act as a filter.
        This produces evolutionary change over time...

Objectives:

Be able to describe the differences between such point mutations as: missense, nonsense, silent, and frameshift mutations. How important might each be in terms of effects on the protein that will be made? Are these mutations in genes or non-genetic regions of the DNA? Are they in introns or in exons?

What is the typical rate of mutational changes seen in eukaryotes? What consequence does this have for the number of cell divisions that can be done in a multicellular organism?

What are transposons and of what parts are they composed? What is the difference between a simple and a composite transposon? What enzymatic activities are needed for transposition to take place? What could be some possible consequences of transposable elements on the control of gene expression?

What are changes that can happen in a chromosome, other than in the expressed genes themselves, that could alter gene expression? What are ways in which the structure of a chromosome can be altered?

Mutations can lead to cancer. Based on what is covered in the text (see pgs. 373-377) be able to describe changes in the controls of several types of genes that typically lead to cancer.

How do the processes of endosymbiosis and polyploidy promote the production of new genetic combinations? Do these processes occur within an individual, within a species, or can they occur between individuals of different species? Some describe organisms like ourselves as chimera (a mixture of several different species). What arguments support this view?

Be able to argue for how mutations are on one hand costly to an individual, but they are also often ultimately beneficial to a species, and helpful in promoting the process of speciation.

For review, see self-quiz questions #3, 4, and 5 of chapter 21.

Needed overheads and items:

Fig. 17.23, types of point mutations
Fig. 17.5, genetic code
Fig. 17.23, types of point mutations
Fig. 17.22, single base pair change, sickle cell anemia
Fig. 16.18, DNA repair mechanisms
Fig. 21.12, unequal crossing over
Fig. 18.19a, (Campbell and Reece, 2005) simple transposon
Fig. 18.17, (Campbell and Reece, 2002) transposon insertion and target sites
Fig. 21.9a, transposon
Fig. 21.9b, retrotransposons
Fig. 21.14, exon reshuffling
Fig. 21.13, gene family
Fig. 18.8, eukaryotic gene and its control regions
Fig. 18.20, genetic changes for oncogene production
Fig. 21.8, McClintock
Fig. 15.15, alternations in chromosome structure
Pennisi etal 2007 - primate tree.jpg
Fig. 15.13, meiotic nondisjunction
Fig. 24.10, autopolyploidy
Fig. 6.9 animal cell
Fig. 25.9, endosymbiosis
Fig. 28.5, (Campbell and Reece, 2002) secondary endosymbiosis
Fig. 24.11, allopolyploidy
Fig. 20.17, (Campbell and Reece, 2005) DNA fingerprinting

Handout:
Handout lecture 17 - mutations.stm
Fig. 18.19, (Campbell and Reece, 2005) simple and composite transposons
Fig. 18.17, (Campbell and Reece, 2002) transposon insertion and target sites

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Biology. Eighth edition. Pages 297-302, 316-318, 344-346, 356-363, 373-377, 432-442, 495-496, and 576-577. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-Biology, 7th edition, figures 18.19, 20.17. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-Biology, 6th edition, Figures 18.17, 28.5. Benjamin Cummings Press. San Francisco, CA.

Han K., M.K. Konkel, J. Xing, H. Wang, J. Lee, T.J. Meyer, C.T. Huang, E. Sandifer, K. Hebert, E.W. Barnes, R. Hubley, W. Miller, A.F.A. Smit, B. Ullmer, M.A. Batzer- 2007-Mobile DNA in old world monkeys: A glimpse through the rehesus monkey genome- Science 316: (4/13) 238-240

Holtcamp W- 2007-Sympathy for the devil. Ideas emerge to save the dying Tasmanian devil- Scientific American 296: (#3, Mar) 27

Pennisi E- 2007-Genomicists tackle the primate tree- Science 316: (4/13) 218-221

Zimmer C- 2006-Evolved for cancer?- Scientific American 296: (#1, Jan.) 68-75

Related issues:

This chapter reviews how to use mutations to study **gene functions**.

> Hartwell L., L. Hood, M.L. Goldberg, A.E. Reynolds, L.M. Silver, R.C. Veres- 2000- Anatomy and function of a gene: Dissection through mutation- Chapter 6, pgs. 179-221, in, <u>Genetics: From Genes to Genomes</u>- McGraw Hill. Boston, MA.

Most species, including *Homo sapiens*, have a high degree of **intraspecific genetic variability**. Here are some articles that relate to human genetic variability.

> Balter M- 2007-Brain evolution studies go micro- Science 315: (3/2) 1208-1211
> Cohen J- 2007-A little gene xeroxing goes a long way- Science 317: (#5844, 9/14) 1483
> Drayna D- 2005-Founder mutations- Scientific American 293: (#4, Oct.) 78-85
> Swaminathan N- 2008-Maverick against the Mendelians- Scientific American 298: (#2, Feb) 86-88

Genetic variations have been found in many species. Here are reports of some means of producing such varation and their consequences. This includes the topics of **retrotransposition**, **pseudogenes**, and others.

> Gerstein M., D. Zheng- 2006-The real life of pseudogenes- Scientific American 295: (#2, Aug) 48-55
> Gonzáles J., D. Petrov- 2009-MITEs - The ultimate parasites- Science 325: (#5946, 9/11) 1352-1353
> Kaessmann H- 2009-More than just a copy- Science 325: (#5943, 8/21) 958-959
> Xiao H., N. Jiang, E. Schaffner, E.J. Stockinger, E. van der Knaap- 2008-A retrotransposon-mediated gene duplication underlies morphological variation in tomato fruit- Science 319: (#5869, 3/14) 1527-1530

Some of the biggest genome rearrangements occur during the formation of the **macronucleus** in **ciliates**. This process involves **transposases** that remove **transposons** from the genome.

> Nowacki M., B.P. Higgins, G.M. Masquilan, E.C. Stwart, T.G. Doak, L.F. Landweber- 2009-A functional role for transposases in a large eukaryotic genome- Science 324: (#5929, 5/15) 935-938

Of course, mutations are needed to induce cancers. Here are some studies of **oncogenes** and the mutations that induce their cancer causing activities. Some of these mutations are due to **errors in DNA repair**.

Barabé F., J.A. Kennedy, K.J. Hope, J.E. Dick- 2007-Modeling the initiation and progression of human acute leukemia in mice- Science 316: (4/27) 600-604

Duesberg P- 2007-Chromosomal chaos and cancer- Scientific American 296: (#5, May) 52-59

Knipscheer P., M. Räschle, A. Smogorzewska, M. Enoiu, T.V. Ho, O.D. Schärer, S.J. Elledge, J.C. Walter- 2009-The fanconi anemia pathway promotes replication-dependent DNA interstrand cross-link repair- Science 326: (#5960, 12/18) 1698-1701

Sjöblom T., S. Jones, L.D. Wood, D.W. Parsons, J. Lin, T.D. Barber, D. Mandelker, R.J. Leary, J. Ptok, N. Silliman, S. Szabo, P. Buckhaults, C. Farrell, P. Meek, S.D. Markowitz, J. Willis, D. Dawson, J.K.V. Willson, A.F. Gazder, J. Hartigan, L. Wu, C. Liu, G. Parmigiani, B.H. Park, K.E. Bachman, N. Papodopoulos, B. Vogelstein, K.W. Kinzler, V.E. Venculescu- 2006-The concensus coding sequences of human breast can colorectal cancers- Science 314: (10/13) 268-274

Trent J.M., J.W. Touchman- 2007-The gene topography of cancer- Science 318: (#5853, 11/16) 1079-1080

Wood L.D., D.W. Parsons, S. Jones, J. Lin, T. Sjöblom, R.J. Leary. D. Shen, S.M. Boca, T. Barber, J. Ptak, N. Silliman, S. Szabo, Z. Dezso, V. Ustyanksky, T. Nikolskaya, Y. Nikolsky, R. Karchin, P.A. Wilson, J.S. Kominker, Z. Zhang, R. Croshaw, J. Willis, D. Dawson, M. Shipitsin, J.K.V. Willison, S. Sukumar, K. Polyak, B.H. Park, C.L. Pethiyagoda, P.V.K. Pant, D.G. Ballinger, A.B. Sparks, J. Hartigan, D.R. Smith, E. Suh, N. Papadopouluos, P. Buckhaults, S.D. Markowitz, G. Parmigiani, K.W. Kinzler, V.E. Velculescu, B. Vogelstein- 2007-The genomic landscapes of human breast and colorectal cancers- Science 318: (#5853, 11/16) 1108-1113

Here is a report of a **missense mutation** that is associated with coronary disease in humans.

Mani A., J. Radhakrishnan, H. Wang, A. Mani, M-A. Mani, C. Nelsen-Williams, K.S. Carew, S. Mane, H. Najmabadi, D. Wu, R.P. Lifton- 2007-LRP6 mutation in a family with early coronary disease and metabolic risk factors- Science 315: (3/2) 1278-1282

This study reports a means to detect **point mutations**.

Colbert T., B.J. Till, R. Tompa, S. Reynolds, M.N. Steine, A.T. Yeung, C.M. McCallum, L. Coma, S. Henikoff- 2001-High-throughput screening for induced point mutations- Plant Physiology 126: 480-484

Not all **silent mutations** have to be for different codons, some can also alter the folding of the mRNA which can affect rates of translation in subtle ways. Here are some examples.

Chamery J.V., L.D. Hurst- 2009-The price of silent mutation- Scientific American 300: (#6, June) 46-53

Kudla G., A.W. Murray, D. Tollervey, J.B. Plotkin- 2009-Code-sequence determinants of gene expression in *Escherichia coli*- Science 324: (#5924, 4/10) 255-258

Frameshifting has been found to occur during translation in mitochondria making different gene products from the same mRNA and introducing errors.

Temperley R., R. Richter, S. Dennerlein, R.N. Lightowers, Z.M. Chzanowska-Lightowlers- 2010-Hungry codons promote frameshifting in human mitochondrial ribosomes- Science 327: (#5963, 1/15) 301

Here is a consideration of **viral mutation rates**.

Flint S.J., L.W. Enquist, V.R. Racaniello, A.M. Skalka- 2004-Evolution and emergence, chapter 20, pgs. 758-802, in Principles of Virology: Molecular biology, pathogenesis, and control of animal viruses- ASM Press. Washington D.C.

Here is a report of the **bacterial mutation rates** that are observed.

Perfeito L., L. Fernandes, C. Mofa, I. Gorda- 2007-Adaptive mutations in bacteria: High rate and small effects- Science 317: (#5839, 8/10) 813-815

Here is a study of the **rate of mutation** seen in a **eukaryote**.

Ossowski S., K. Schneeberger, J.I. Lucas-Liedó, N. Warthmann, R.M. Clark, R.G. Shaw, D. Weigel, M. Lynch- 2010-The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*- Science 327: (#5961, 1/1) 92-94

These articles find that **chromatin structure** influences mutation rates in our chromosomes.

Semple C.A.M., M.S. Taylor- 2009-The structure of change- Science 323: (#5912, 1/16) 347-348

Sasaki S., C.C. Mello, A. Shimada, Y. Nakatani, S-i. Hashimoto, M. Ogawa, K. Matsuchima, S.G. Gu, M. Kasahara, B. Ahsan, A. Sasaki, T. Saito, Y. Suzuki, S. Sugano, Y. Kohara, H. Takeda, A. Fire, S. Morishita- 2009-Chromatin-associated periodicity in genetic variation- Science 323: (#5912, 1/16) 401-404

We are used to thinking of mutations as being distinct separate events that do not influence each other, but that is not always the case. This article notes that mutations in certain regions of a chromosome can alter the **rates of mutation** in other parts of the chromosome. The **positions of chromosomes** also is found to correlate with different rates of mutation.

Mani R-S., S.A. Tomlins, K. Callahan, A. Ghosh, M.K. Nyati, S. Varambally, N. Palanisamy,
A.M. Chinnaiyan- 2009-Induced chromosomal proximity and gene fusions in prostate cancer- Science 326: (#5957, 11/27) 1230

Sureshkumar S., M. Todesco, K. Schneeberger, R. Harilai, S. Balasubramanian D. Wrigel- 2009-A genetic defect caused by a triple repeat expansion in *Arabidopsis thaliana*- Science 323: (#5917, 2/20) 1060-1063

This article reports on the insertion of new **introns** into a gene in a crustacean.

Li W., A.E. Tucker, W. Sung, W.K. Thomas, M. Lynch- 2009-Extensive, recent intron gains in *Daphnia* populations- Science 326: (#5957, 11/27) 1260-1262

DNA sequencing has become so facile that they can now estimate the rate of **intergenerational mutations** in a family.

Roach J.C., G. Glusman, A.F.A. Smit, C.D. Huff, R. Hubley, P.T. Shannon, L. Rowen, K.P. Pant, N. Goodman, M. Bamshad, J. Shendure, R. Drmanac, L.B. Jorde, L. Hood, D.J. Galas- 2010-Analysis of genetic inheritance in a family quartet by whole-genome sequencing- Science 328: (#5978, 4/30) 636-639

BIO 108   2010
Day 9, Lecture 24, Title: Recombinant DNA II.
Text Readings: Campbell et al. (2008), pgs. 561-564, and chapter 20.

Topics to cover:

> **Where we left off...**
> **3) Create vector with desired DNA**
> > **Plasmids**
> > **Artficial Chromosomes**
> **4) Introduce the DNA into test organisms and find it**
> > **Transformation**
> > **Transduction**
> > **Microinjection**
> **5) Check functionality of DNA**
> > **Hybridization**
> > **Reporter Gene uses**
> > **Southern, Northern, Western Blots**
> **Other Issues/Options**
> > **cDNA**
> > **DNA Microarrays**
> > **DNA Libraries**
> > **Gene "Therapy"**

Where we left off...
Steps needed to be able to create and use recombinant DNA
> 1) Isolate desired DNA
> 2) Produce more of the same DNA
So have isolated a candidate desired gene. Have made more of it for use.
Now, will consider the next steps...
> 3) Create vector with desired DNA
> 4) Introduce the DNA into test organisms and find it
> 5) Check functionality of DNA

3) Create vector with desired DNA
Vector: a construct used to deliver DNA into a cell and have it expressed there.
So what is needed in a vector? It not only has to hold our DNA, but hold it in a functional way.
> The vector may have features that help to get the DNA into the cell.
> So have expression issues as well as delivery issues.

1

If want gene expression, then need...

        promoter, regulatory elements?   This can control which tissue or cell type...

        exon/intron issues   What processing demands?

        post-translational needs?

            cofactors, other subunits... etc

If want replication?

        ORI region, telomeres

If want it to incorporate into host DNA?

        Ability to act as a transposon?   What site(s) in host will it insert into?

Plasmids

Use of plasmids as vectors is common when working with bacteria...

        Fig. 20.4 Cloning using plasmids

        What is a plasmid? (this is being used in the lab exercises...)

        What is needed for a bacterium to replicate a plasmid?

            recall viral lysogenic cycle, plasmid integration

            origin of replication

            space for other genes, can put in one we want, or several all at once...

                most plasmids can carry one to a dozen genes

            need restriction enzymes and DNA ligase

                (Where have we seen DNA ligase before?)

Artficial Chromosomes

In what type of organism will it be expressed? Does it normally have plasmids?

If not, then can use chromosomes we make.

Use of BACs and YACs: Bacterial Artificial Chromosome, Yeast Artificial Chromosome

            even have Human Artificial Chromosomes now...

        can move more than one gene at a time... can hold hundreds to thousands

            so often move sets of genes for entire pathways at once...

So now we have our gene(s) put into a vector. Next need to get it into an organism.

4) Introduce the DNA into test organisms and find it

        How to get your DNA into an organism?

        And how to find the one individual in a million that took it up properly?

Transformation

Transformation, can use naked linear DNA or circular DNA, but very inefficient...

        Recall Fred Griffith's expt. with R and S strains, used transformation

        Fig. 16.2, bacterial transformation

        Can expose cell to naked DNA, uptake and integration happens, but is rare

            being rare is not often much of a problem

            can expose hundreds of millions of individuals at once

Ways to promote DNA uptake?
    Use of transformation "competent" bacteria, at right stage
        strains of cells that take up DNA better?
        transformation "competant"?
        so they have deployed molecular machinery to take up DNA
    use heat shock
    polyethylene glycol treatment
    Calcium ion manipulation
    Electroporation

Transduction
Transduction uses virus to carry DNA, so need to make viral-sized DNA
    Fig. 18.16, (Campbell and Reece, 2005) transduction
    DNA is surrounded by protein coat,
        functions to insert DNA into viral-specific target cell...
        sometimes gets host DNA instead of viral DNA
        so can use this to move our DNA
        can purchase viral proteins
        assemble spontaneously arround DNA

Microinjection
Microinjection uses a solution of your DNA in a glass micropipette
    Fig. 20.2, (Campbell and Reece, 2002) microinjection
    can put it directly into host cell nucleus
    Good for one cell at a time... Typically 100s of attempts give one successful uptake...

Other methods of getting the genetic material into the target cells exist...

So now have our desired gene(s) in organism. Is it functional?

5) Check functionality of DNA

       "Functioning" depends on context of DNA you are studying...

       What happens to foreign DNA once in bacterial or eukaryotic cells?

              most will be degraded

              only part integrated or replicated?

              some might be integrated, but will not be expressed?

              some may be integrated and expressed but protein is not functional?

       So much needs to be checked here.

Hybridization

       Is the gene of interest there in the cell?

       Can test this via in situ hybridization

       Plate out individual cells

              Fig. 20.5, (Campbell and Reece 2005) probe for cloned gene

              grow into colonies, this produces "cloned" DNA

              take print on sterile filter paper

              lyse and probe with Nucleic acid probe for gene

              probe must be specific, and detectable

                     (Why won't ethidium bromide work here?)

              grow those colonies that are of interest

              This tells you if the DNA is in there

Reporter Gene uses

How to find those few cells that have taken up your DNA?

       Use of reporter gene-systems

              The changes in phenotype they code for tell us about the system

       Fig. 20.4, Cloning using plasmids

       Some reporter gene-systems use genes for antibotic resistance

              others use gene for B-galactosidase activity

                     this can cleave X-gal, producing a blue color...

              if these expressed then maybe other genes

                     inserted and expressed as well

                     Can be turned on, off, or in new combinations

                          Gain-of-function, loss-of-function systems

                     Here one stays on (ampicillin resistance: gain-of-function)

                     other turns off (lacZ gene: loss-of-function)

       When in the new cell these alter the cell's phenotype

              ability to resist an antibiotic is a phenotypic trait

              ability to metabolize X-gal is a metabolic trait, a phenotype

Southern, Northern, Western Blots

Check functionality of DNA

> Recall that can use Southern Blotting to check for presence of DNA in sample from cell
>> But if in cell it may or may not work the way you wish?

> Now can study whether DNA and its products works

> Northern Blotting uses isolated RNAs, which implies gene expression is occurring
>> Can isolate mRNA and see if gene is expressed
>>> if know gene, can probe RNA with its complement
>>>> separate RNA on gel
>>>> blot on to paper
>>>> probe with complementary RNA probe
>>> this can tell you if the gene is used in transcription

> Western Blotting tells you if a protein is made
>> But is protein made in an active and functional form?
>>> Need to isolate protein and have assay for its presence.
>>> Use of antibodies to bind to selected protein
>>>> Western Blot
>>>>> separate proteins on gel, blot onto paper
>>>>> use specific antibodies as probes

Recall steps in the process

> Fig. 20.2, steps in cloning DNA and its use

Other Issues/Options

Options and other issues related to this process:

> Can start with DNA, as has been outlined here
>> this gives ability to clone and study regions other than just genes:
>>> promoters, introns, regulatory sequences, etc...

cDNA

Can start with mRNA

> make complementary DNA (cDNA)

> Fig. 20.6, cDNA

> Use reverse transcriptase (where have we seen this before?)
>> this gives information on
>>> mRNA, so works for currently expressed genes
>>>> in specific cells/tissues/conditions/developmental stage
>> Note use of primer, finds poly-A tail. So good for getting mRNAs?

> Use DNA polymerase
>> need primer, but this may not be broad, could limit which ones used?

DNA Microarrays

Microarray analysis

    Fig. 20.15, DNA microarray

        each well has distinct probe bound to wall of the well

        so can examine gene expression globally, 1000s all at once!

    Fig. 20.1, DNA microarray

    This allows probing of isolated DNAs or mRNAs from a sample

        so can see which genes are present, or

        are being expressed at one stage of development or in one tissue type

DNA Libraries

    Fig. 20.5, DNA library

    This is what is done if studying an entire genome

    Instead of using specific DNA use all restriction products

        put each type into a distinct bacterial cell and let it clone it

    So have all of DNA from specimen in plasmids in bacterial cells

        or can be in viral particles

    Easy to grow and survey.

        a way to store the samples, as can grow up more DNA for later use

    Various restriction enzymes give various pieces from same genome

    So get various libraries

    This can be useful in seqencing a genome.

        Both use fragments put into libraries, and then isolated for sequencing

        use of probes and sequencing to sequence sections

        This is one approach for sequencing an unknown genome.

Gene "Therapy"

We now can insert individual genes, or sets of genes into human cells

    either somatic adult cells, or gametes, or zygotes

Or we can alter promoters or expression of existing genes

"Gene Therapy" insertion of genes into humans.

    Fig. 20.22, gene therapy

    has resulted in several deaths....

Several assumptions and preconditions here.

    We must understand what genetic factors need to be altered

    The gene causes no harm, we know where it inserts,

        what cells will get it, whether it is inherited or not..

    some of these assumptions are rarely tested...

First example:

 Beardsley (2000) and Wenner (2008) popular articles

 Jesse Gelsinger died on September 17, 1999

   Jesse Gelsinger - Wenner 2008.jpg

 four days after he was given a gene therapy treatment

  an "overwhelming" immune reaction

  engineered adenovirus vector given to his liver

 His disease was OTCD, ornithine transcarbamylase deficiency,

  it leads to accumulation of ammonium in the blood.

 Adenovirus. Some of these are known to lead to illness

  but the one used had genes replaced

  the coat was used as a delivery system.

  Evidence exists that the modified adenovirus

   experienced variation over time. So there might

   be varients in the treatment given.

 Others who had used this experimental system had failed to report the death of monkeys who

  had been given similar modified adenovirus.

 Many safety protocols not followed

  Earlier patients in OTCD treatment had been given

   lower doses, and they had experienced liver

   problems.

  original protocol called for test of treatment on

   patients with a lower blood ammonium level than

   Gelsinger had when he was treated, less stress

  and it called for women only as patients as they would

   be more resistant to complications.

Second example: Boyce (2002) popular article

 use human gene therapy to swap in a gene to bone marrow cells of children with immune

  deficiency disease (SCID is the "bubble boy" disease).

 10 children were treated in Paris.

 But one of the earlier treated children has developed leukemia-like illness. It is feared the

  gene swap promoted this cancer.

 So FDA has suspended trials of this treatment in U.S. Another set back to gene therapy.

 Uses crippled viruses to get genes into cells. But where the virus inserts the gene is not

  controlled, so it might induce new problems depending on where inserted.

 In the case of this boy the gene was inserted near a gene known to be involved in human

  leukemias. It may have altered its expression?

Gene therapy has been used more in the past few years as better/safer viral vectors are found.

So can put genes into organisms to create pheotypes we might want

 Pg. 389 Mouse with jellyfish gene.jpg (Freeman 2008)

 Human cloning NYTimes magazine.jpg

 Last step: Apply for a patent!

Objectives:

Be able to describe several options for getting foreign DNA into a host cell. What types of vectors are available that can carry the DNA into the cell? What various methods can be used to get DNA into a cell? Be able to describe some advantages and disadvantages of each method and vector type. How is the uptake of the DNA detected? Be able to describe the use of reporter genes (see fig. 20.4), how each alters the phenotype of the cell that expresses them, and what sort of information each type of reporter gene provides us.

Getting the DNA into the cell is not enough, what else must happen? What could go wrong if you attempt to express a eukaryotic gene in a bacterial cell? What is needed to carry out Northern and Western blotting techniques, and what does each of these types of blotting tell you compared to what Southern blotting could tell you?

What are YACs and BACs and what do they allow molecular biologists to do that simple transformation or plasmid-based systems can not?

What is a DNA library, and for what it is used? How would it be prepared? What can a DNA microarray allow you to do, and how does it detect DNA? What are some advantages and disadvantages of working with DNA versus mRNA as the starting material? What is cDNA, and what would it tell you that you could not easily determine if you worked directly with DNA?

What is gene "therapy" and what is it attempting to do, and what are some of techniques upon which it depends?

For review, see self-quiz questions #2, 8, 9 and 12 of chapter 20.

Needed overheads and items:

Fig. 20.4 Cloning using plasmids
Fig. 16.2, bacterial transformation
Fig. 18.16, (Campbell and Reece, 2005) transduction
Fig. 20.2, (Campbell and Reece, 2002) microinjection
Fig. 20.4 Cloning using plasmids
Fig. 20.5, (Campbell and Reece, 2005) probe for cloned gene
Fig. 20.2, steps in cloning DNA and its use
Fig. 20.6, cDNA
Fig. 20.15, DNA microarray
Fig. 20.1, DNA microarray
Fig. 20.5, DNA library
Fig. 20.22, gene therapy
Jesse Gelsinger - Wenner 2008.jpg
Pg. 389 Mouse with jellyfish gene.jpg (Freeman 2008)
Human cloning NYTimes magazine.jpg

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Beardsley T- 2000-Gene therapy setback- Scientific American 282: (#2) 36-37.

Boyce N- 2002-Too high for a cure?- U.S. News & World Report 133: (#14, Oct. 14) 34.

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      R.B.Jackson- 2008-Biotechnology. Chapter 20 and pgs. 561-564. Biology.
      Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-Biology, 7th edition, Figures 18.5 and 20.5. Benjamin
      Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-Biology, 6th edition, Figures 20.2. Benjamin
      Cummings Press. San Francisco, CA.

Freeman S- 2008-Biological Science, 3rd edition. Figure 19.18, and pg. 389. Pearson
      Benjamin Cummings. San Francisco, CA.

New York Times Magazine, Feb. 4, 2001, section 6.

Wenner M- 2008-Regaining lost luster- Scientific American 298: (#1, Jan) 18-20

Related issues:

An entire **bacterial artificial chromosome**, not merely splicing together of existing pieces, has been created and put into a bacterium. So chromosomes of megabase sizes can now be made.

Endy D- 2008-Reconstruction of the genomes- Science 319: (#5867, 2/29) 1196-1197

Gibson D.G., G.A. Benders, C. Andrews-Pfannkoch, E.A. Denisova, H. Baden-Tillson, J. Zaveri, T.B. Stockwell, A. Brownley, D.W. Thomas, M.A. Algire, C. Merryman, L. Young, V.N. Noslov, J.I. Glass, J.C. Venter, C.A. Hutchison III, H.O. Smith- 2008-Complete chemical syntehsis, assembly, and cloning of a *Mycoplasma genitalium* genome- Science 319: (#5867, 2/29) 1215-1220

Which means that they now can **move entire genomes** between species!

Lartigue C., S. Vashee, M.A. Algire, R-Y. Chuang, G.A. Benders, L. Ma, V.N. Noskov, E.A. Denisova, D.G. Gibson, N. Assad-Garcia, N. Alperovich, D.W. Thomas, C. Merryman, C.A. Hutchison III, H.O. Smith, J.C. Venter, J.I. Glass- 2009-Creating bacteria strains from genomes that have been cloned and engineered in yeast- Science 325: (#5948, 9/25) 1693-1696

Lartigue C., J.I. Glass, N. Alperovich, R. Piéper, P.P. Parmar, C.A. Hutchison III, H.O. Smith, J.C. Venter- 2007-Genome transplantation in bacteria: Changing one species into another- Science 317: (#5838, 8/3) 632-638

Pennisi E- 2009-Two steps forward for synthetic biology- Science 325: (#5943, 8/21) 928-929

One type of **vector** of genes is a plasmid transmitted in a bacterial phage, this is called a **phasmid**.

Castagnoli L., G. Cesareni, S. Brenner- 1982-The phasmid as a tool for plasmid genetics. I. Fine structure of the β-lactamase gene- Genetical Research 40: 217-231

Nucleic acid hybridization is the basis of many techniques. Here is an example of its use.

Ke Y., S. Lindsay, Y. Chang, Y. Liu, H. Yan- 2008-Self-assembled water-soluble nucleic acid probe tiles for label-free RNA hybridization assays- Science 319: (#5860, 1/11) 180-183

Amplifying DNA via PCR introduces errors. This study turns this into an advantage by creating **yeast GMOs** with various PCR products in an attempt to produce new genetic combinations with desired metabolic traits, in this case ethanol production!

Alper H., J. Moxley, E. Nevoigt, G.R. Fink, G. Stephanopoulos- 2006-Engineering yeast transcription machinery for improved ethanol tolerance and production- Science 314: (12/8) 1565-1568

Here are some articles relating to the uses and problems of **gene therapy** which recently has been attempted again after some earlier fatal experiments. It even has been tried by atheletes (i.e. **gene doping**) who try to genetically modify themselves!

Cartier N., S. Hacein-Bey-Abina, C.C. Bartholomae, G. Veres, M. Schmidt, I. Kutschera, M. Vidaud, U. Abel, L. Dal-Cortivo, L. Caccavelli, N. Mahlaoui, V. Kiermer, D. Mittelstaedt, C. Bellesme, N. Kahiou, F. Lefrère, S. Blanche, M. Audit, E. Payen, P. Lebouich, B. l'Homme, P. Bouguères, C. VonKalle, A. Fischer, M. Cavazzana-Calvo, P. Aubourg- 2009-Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy- Science 326: (#5954, 11/6) 818-823

Friedmann T., O. Rabin, M.S. Frankel- 2010-Gene doping and sports- Science 327: (#5966, 2/5) 647-648

Guo J., H. Xin- 2006-Splicing out the west?- Science 314: (11/24) 1232-1235

Kaiser J- 2009-β-Thalassemia treatment succeeds, with a caveat- Science 326: (#5959, 12/11) 1468-1469

Kaiser J- 2008-Two teams report progress in reversing loss of sight- Science 320: (#5876, 5/2) 606-607

Kaiser J- 2007-Gene transfer an unlikely contributor to patient's death- Science 318: (#5856, 12/7) 1535

Kaiser J- 2007-Questions remain on cause of death in arthritis trial- Science 317: (#5845, 9/21) 1665

Kaiser J- 2007-Death prompts a review of gene therapy vector- Science 317: (#5838, 8/3) 580

Kaiser J- 2005-Retroviral vectors: A double-edged sword- Science 308: (6/17) 1735-1736

McCormack M.P., L.F. Young, S. Vasudevan, C.A. de Graaf, R. Codrington, T.H. Robbitts, S.M. Jane, D.J. Curtis- 2010-The Lmo2 oncogene initiates leukemia in mice by inducing thymocyte self-renewal- Science 327: (#5967, 2/12) 879-883

Naldini L- 2009-A come back for gene therapy- Science 326: (#5954, 11/6) 805-806

Shaffer C.H- 2003-You can change your genes- Analog: Science Fiction and Fact- 123: (#9, Sept) 46-53

Sweeney H.L- 2004-Gene doping- Scientific American 291: (#1) 62-69

Wenner M- 2009-Tribulation of a trial- Scientific American 301: (#3, Sept) 14-15

Yam P- 2003-Insulin from bone marrow- Scientific American 288; (#5) 33

They have managed to sequence **DNA** from the bones of **neandertals** and other hominins!

Balter M- 2010-Ancient DNA from Siberia fingers a possible new human lineage- Science 327: (#5973, 3/26) 1566-1567

Recently a court has overturned some **patents** on **human genes**.

Marshall E- 2010-Cancer gene patents ruled invalid- Science 328: (#5975, 4/9) 153

We use DNA technologies in part, to study the **genomes** of various species. Here are a few reports of some of the recently completed **genome sequences** and studies of them.

Carlton J.M., R.P. Hirt, J.C. Silva, A.L. Delcher, M. Schatz, Q. Zhao, J.R. Wortman, S.L. Bidwell, U.C.M. Alsmark, S. Besteiro, T. Sicheritz-Ponten, C.J. Noel, J.B. Dacks, P.G. Foster, C. Simillion, Y. Van de Peer, D. Miranda-Saavedra, G.J. Barton, G.D. Westrop, S. Müller, D. Dessi, P.L. Fiori, Q. Ren, I. Paulsen, H. Zhang, F.D. Bastida-Corcuera, A. Simoes-Barbosa, M.T. Brown, R.D. Hayes, M. Mukherjee, C.Y. Okumura, R. Schneider, A. J. Smith, S. Vanacova, M. Villalvazo, B.J. Haas, M. Pertea, T.V. Feldblyum, T.R. Utterback, C-L. Shu, K. Osoegawa, P.J. de Jong, I. Hrdy, L. Horvathova, Z. Zubacova, P. Dolezal, S-B. Malik, J.M. Logsdon Jr., K. Henze, A. Gupta, C.C. Wang, R.L. Dunne, J.A. Upcroft, P. Upcroft, O. White, S.L. Salzberg, P. Tang, C-H. Chiu, Y-S. Lee, T.M. Embley, G.H. Coombs, J.C. Mottram, J. Tachezy, C.M. Fraser-Liggett, P.J. Johnson- 2007-Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*- Science 315: (1/12) 207-212

Chadee D.D., P. Kitayapong, A.C. Morrison, W.J. Tabachnick- 2007-A break through for global public health- Science 316: (#5832, 6/22) 1703-1704

Clark R.M., G. Schweikert, C. Toomajian, S. Ossowski, G. Zeller, P. Shinn, N. Warthmann, T.T. Hu, G. Fu, D.A. Hinds, H. Chen, K.A. Frazer, D.H. Huson, B. Schölkopf, M. Nordborg, G. Rätsch, J.R. Ecker, D. Weigel- 2007-Common sequence polymorphisms shaping genetic diversity in *Arabidopsis thaliana*- Science 317: (#5836, 7/20) 338-342

Cohen J- 2007-Relative differences: The myth of 1%- Science 316: (#5833, 6/29) 1836

Feuillet C., K. Eversole- 2009-Solving the maze- Science 326: (#5956, 11/20) 1071-1072

Ghedin E., S. Wang, D. Spiro, E. Caler, Q. Zhao, J. Crabtree, J.E. Allen, A.L. Delcher, D.B. Guiliano, D. Miranda-Saavedra, S.V. Angiuoli, T. Creasy, P. Amedeo, B. Haas, N.M. El-Sayed, J.R. Wortman, T. Feldblyum, L. Tallon, M. Schatz, M. Shumway, H. Koo, S.L. Salzberg, S. Schobel, M. Pertea, M. Pop, O. White, G.J. Barton, C.K.S. Carlow, M.J. Crawford, J. Daub, M.W. Dimmic, C.F. Estes, J.M. Foster, M. Ganatra, W.F. Gregory, N.M. Johnson, J. Jin, R. Komuniecki, I. Korf, S. Kumar, S. Laney, B-W. Li, W. Li, T.H. Lindblom, S. Lustigman, D. Ma, C.V. Maina, D.M.A. Martin, J.P. McCarter, L. McReynolds, M. Mitreva, T.B. Nutman, J. Parkinson, J.M. Peregrín-Alvarez, C. Poole, Q. Ren, L. Saunders, A.E. Sluder, K. Smith, M. Stanke, T.R. Unnasch, J. Ware, A.D. Wei, G. Weil, D.J. Williams, Y. Zhang, S.A. Williams, C. Fraser-Liggett, B. Slatko, M.L. Blaxter, A.L. Scott- 2007-Draft genome of the filarial nematode parasite *Brugia malayi*- Science 317: (#5845, 9/21) 1756-1760

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BIO 108   2010
Day 10, Lecture 25, Title: Darwinian Evolution.
Text Readings: Campbell et al. (2008), Chapter 22.

Topics to cover:

> **Pre-Darwinian Views and Contributions from other Fields**
> > **Economists**
> > **Geology**
> > **Taxonomy**
> > **Natural Philosophers**
> **What Charles Darwin did and knew**
> > **Alfred Russell Wallace**
> **Darwin and Wallace's Model of Speciation**
> > **What he adopted from other fields**
> > **Observations this model explains**
> > **Additional conditions of the model**
> > **Problems Darwin encountered with his model**

Pre-Darwinian Views and Contributions from other Fields
> What was the view before Charles Darwin's time? (These are not obvious to us...)
> Young Earth
> > just tens of thousands of years old
> > imagine how that limits conceptional options...
> > > how to account for the grand canyon? Or a flood plane? Or life?
> Catastrophism
> > attempts to account for observed gelolgical features by special events
> > but what were fossils? Demons turned to stone?
> Special creation for each species
> > possibility of spontaneous generation was considered, soil in area mattered?
> > similar soil/climate gave rise to similar species in similar climates.
> "Type" species, of limited ability to vary from an ideal form, do not go extinct...
> > Dogs have an ideal "type" to which they are constrained... though can vary a bit...

> **Economists**
> New views brought forward, one view from economists
> Malthus, "Essay on the Principle of Population"
> > argument on human population growth and food production
> > > high reproduction rate, yet population is often observed to be static
> > > food production not able to keep up with potential population growth
> > > so many offspring must die, suggesting a struggle for existence

Geology
This field also brought new views forward.
Fig. 22.2, historical aspects about Darwinism
      this figure has a good summary of historical considerations
Hutton, gradualism (Repcheck, 2003)
      notion of "deep time" was developed by him, but not accepted in his day
Lyell, uniformitarianism
      he found good evidence for above, and convinced geologists
      Fig. 22.3, sedimentary rock and fossils
The idea of an ancient earth opened the way to use fossils as a relative indicator of time
      also opened up the possibility of slow processes producing large changes...

Taxonomy
A critical mass of information in biology was accumulated by taxonomists
Linnaeus, and others did much to organize this information to make it useful
      grouped species by similar traits, binomial system...
This allowed for views across groups of life, making it possible to detect similarities

Natural Philosophers
Natural Philosophers
      In 18th century there were no "scientists" and no scientific profession...
      Were no trained biologists, but approached life from traditions of philosophy
            "Vestiges" and other publications were done by these natural philosophers.
      Style of argument, often ideas presented without a supporting body of evidence?
      One example was Charles Darwin's own grandfather, Erasmus Darwin
            Erasmus Darwin.jpg (King-Hele, 1999)
            had poems on nature, suggesting speciation had occurred
      None of these people had ever tried to identify a species, but talked alot
            net effect was to tar the whole topic with disrepute...?
            E. Darwin's conjectures became called "Darwinizing" as a prejorative
Lamarck
      Put forward a view of speciation
      But given the view of a young earth had to propose a very rapid mechanism
      Fig. 22.x4, (Campbell and Reece, 2002) Lamarck
      organized fossil record by similarities of traits
      Proposed inheritance of acquired traits
      What is the classical example of Lamarckism?
            Giraffe neck, but he never made it! (Gould, 1998)
            French vs. British science, French viewed as a radical society...
            no mechanism of how to pass on genetic information...
            obvious tests refuted it... (see Gould, 2000)

So a mass of information without a unifying theme, specific details, or mechanism...

What Charles Darwin did and knew
Charles Darwin, and his trip.   Beagle as a research vessel.
        Not clear what to do with his life. Doctor? Naturalist? Clergy?
                Dr Robert Darwin Keynes 2003.jpg
        First he had to convince his father to let him take the trip, did this with help of his uncle
                Dr. Darwin paid for Charles' expenses for the trip...
Voyage of the Beagle, as a gentleman's companion.   offended the ship's surgeon...
        Fig. 22.5, Voyage of the Beagle
        South American rain forest, density of life, and close interactions
        Fossils found in Argentina
                Macraucheria fossil collected by Darwin - Keynes 2003.jpg
        Earthquake effects witnessed in the Andes
                read Lyell's Principles of Geology text
                found marine fossils at peak of Andes, confirmed to him an ancient earth...
        Collected South American specimens
                skins, fossils, preserved in "spirits of wine"
        Then went to Galapagos Islands, for reprovisioning and survey of coasts
                similar to S.American areas, but different flora and fauna, this was a puzzle
                accepted idea at the time was similar species in similar habitats
                collected his finches, failed to note all the locations....
                        Darwins finches - Keynes 2003.jpg
                reports of differences in turtles from island to island
        Specimens shipped back to Britian along with letters, some published
        When returned to Britian had specimens to study and farm out for analysis
                Richard Owens examined his fossils
                Other biologists examined other specimens,
                        this strengthened his connections to British biologists
        Private work on the "species question" was begun
                began breeding studies, studies of artificial selection
                        made extensive use of a wide variety of sources of information
                by 1844 had ideas on speciation, planned to have them published after his death!
                        due to "Vestiges" publication he was afraid of the reaction to his ideas
        Began to collect information on species variation
                studied barnacles, became an authority in systematics (Stott, 2003)

        Alfred Russell Wallace
Wallace's letter to Darwin, June 18, 1858
        Fig. 22.x5, (Campbell and Reece, 2002) Wallace
Wallace was not independently wealthy, was a professional naturalist, surveyor, etc
        Was collecting specimens in South East Asia, and selling them back in Europe
        Also corresponded with Darwin, supplied Darwin with specimens for his barnacle studies

So asked Darwin to pass his paper on to the Linnean Society for publication
Darwin's and Wallace's papers were both published by
Linnean Society, July 1, 1858, no excitement
(neat book on Wallace: Slotten, 2004)
Wallace accepted Darwin's prior and superior claim to the idea of speciation
but the fact that Wallace also came up with it suggests the time was ripe?

Darwin and Wallace's Model of Speciation
Nov. 21, 1859, Published his book, big excitement
Fig. 22.00, (Campbell and Reece, 2002) OriginOfSpecies
(For more on the excitement see Gould's essay "Knight takes Bishop", 1991)
a set of well reasoned arguments backed up by a mass of documented observations
Some items he noted that must exist for his model to work, these are testable to various degrees...
Variation amongst individuals of a species (population-thinking, a new approach)
a paradigm shift from the old view of "type" species where variation did not matter
some variation is more "fit" for survival to reproduce than other variations
he had no idea of the source of this variation, just that it existed...
this variation is heritable
he never came up with a mechanism for heritability in his time...
Excess offspring, that are less fit, tend to die off more and so contribute less to the line
this is population-level thinking, a new way of viewing things...
Over long periods of time (millions of years) this alters a species, resulting in speciation
so speciation is a consequence of these features of life.
Darwin's model is one of speciation from existing species.

What he adopted from other fields
He argued for gradual change vs. sudden special creation
this mimics geologist arguments
If geologists can argue for slow processes created the Andes,
then biologists could argue for slow processes leading to speciation?
rejected earlier proposals of rapid speciation suggested by others
as in "hopeful monsters",
and so the absence of these transition stages is no longer a problem
He argued for excess population and limited supplies (population thinking)
as from Malthius, note his argument of elephants
Choose elephants as the slowest reproducing species he knew of
by simple calculations showed how elephants fit Malthius' arguments
implying the existence of a struggle for resources
This allowed Darwin to argue for natural selection,
with high degree of competition between species and individuals
for limited resources
idea of "wedges" to fill limited niches, competition driven

Natural selection as a driving "force"
        made close analogies to artificial selection
but rarely strong, a constant weak selection was what Darwin had in mind
Natural selection acts as a filter, applied to the variation to result in change
        in the population as a whole (not in an individual...)

Observations this model explained
He also proposed a common, or a few, lines of ancestory for life on Earth.
This would account for the similarities seen...
        Fig. 22.17, homologous structures, forelimbs
        With taxonomic knowledge could look for these implied homologies
        So the way the taxonomists associated species might reflect
            relationships between the species....
Could look at populations of related species and infer the selection each receives
        Fig. 22.13, guppy divergence in different ponds
        if differences in environment   result in different natural selection
        can have speciation
            as seen in his finches and these fish...
Could then make a model of lineages of life, and estimate their relatedness
        Fig. 22.8, descent with modification
        concept of homologies between species now possible
        Descent with modification implies a common ancestor, so phylogenetic trees
            Fig. 22.7, Darwin's notebook
            Fig. 26.21, three domains of life
With a model for speciation, this raised issue of extinction
        earlier view of no speciation also implied no extinction of species
        with a means of new species being formed, then could accept extinction of species
        This gives a new role and importance to information from fossils...
        Can compare fossils to current species, another way to test lineages
        However in Darwin's time the knowledge of fossils was poor,
            so few tested his model this way, though we often do so today...

Additional conditions of the model
There are some conditions that must exist that Darwin did NOT state:
        Earth must exist (so his model does NOT cover origin of the universe)
        Life must exist (so it also is NOT meant to account for the origin of life)
            Darwinian evolution is a model for speciation from existing species
                this decouples evolution from spontaneous generation....
                (Note distinct meanings of "evolution" to biologist, astronomer, etc...)
        Species must be distinct entities at some level
            their variation must not typically be acquired, some means of heredity
        Variation is mainly inherited down through time in distinct lineages of life,
we call this vertical flow of genetic information, horizontal flow of genes must be rare...

Problems Darwin encountered with his model

What he did not know/holes in the model during his time...
      no mechanism of inheritance or genetics was known
            so genetics, DNA, genes, all not known...
            a major hole
            Darwin most likely did not read Mendel's paper
      no connecting links from fossil record in his time, as poorly studied
            argued that fossil record is inadequate, and it was at his time...
      Argued that process of speciation was slow, so not directly observable?
            suggested speciation in hundreds of thousands to millions of years
            this makes it difficult to directly test the model, so had to test it indirectly...
      age of the Earth was not established
            Darwin assumed lots of "deep time"
            Challenge from Lord Kelvin, a physicist
                  projection of age of earth from thermal measurements
                      suggested not even a million years old!
                  did not know about radiological heat
            one of the few times that the biologists
                  told the physicists to go back and check it...

Darwinian Evolution
      A model for speciation: The formation of new species from existing species.
      Based on variation and natural selection, using a population level of thinking
      Our modern models of evolution have been modified from this starting point
            (Similar to how genetics has grown from a Mendelian base...)
            we will cover some of the modifications in a later lecture.

Objectives:

Be able to describe the Darwinian view of evolutionary theory. What conditions must be met, and what are some assumptions made, by Darwin's model of speciation? What did Darwin view as the major mechanism leading to speciation? What predictions follow from Darwinian evolution that would not have been expected otherwise?

What were some of the biological and geological views that preceeded Darwin's model and how did they influence his model? What ideas from geology and economics did Darwin incorporate into his model? What were some of the scientific views before Darwin's time that limited thinking about the formation of new biological species? What were some concepts that became evident once Darwin's model was produced that would not have been much considered before?

Understand the argument that while species have and continue to evolve our biological models of that evolution are incomplete. What were some major aspects of Darwinian evolutionary theory that can be argued to have been incomplete or strongly in doubt at the time he proposed it?

For review, see self-quiz questions #1, 4, 5, 6 of chapter 22.

Needed overheads and items:

Fig. 22.2, historical aspects about Darwinism
Fig. 22.3, sedimentary rock and fossils
Erasmus Darwin.jpg (King-Hele, 1999)
Fig. 22.x4, (Campbell and Reece, 2002) Lamarck
Dr Robert Darwin - Keynes 2003.jpg
Fig. 22.5, Voyage of the Beagle
Macraucheria fossil collected by Darwin - Keynes 2003.jpg
Darwins finches - Keynes 2003.jpg
Fig. 22.x5, (Campbell and Reece, 2002) Wallace
Fig. 22.00, (Campbell and Reece, 2002) OriginOfSpecies
Fig. 22.17, homologous structures, forelimbs
Fig. 22.13, Guppy divergence
Fig. 22.8, descent with modification
Fig. 22.7, Darwin's notebook
Fig. 26.21, three domains of life

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Descent with modification: A Darwinian view of life. Chapter 22. Pages 452-467. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-Biology, 6th edition, Figures 22.0, 22.x4, 22.x5. Benjamin Cummings Press. San Francisco, CA.

Gould S.J- 2000-A tree grows in Paris: Lamarck's division of worms and revision of nature. Pgs. 115-143. In, The lying stones of Marrakech. Harmony Books, N.Y., N.Y.

Gould S.J- 1998-The tallest tale. Pgs.301-318. In, Leonardo's mountain of clams and the diet of worms. Three Rivers Press, N.Y., N.Y.

Gould S.J- 1991-Knight takes Bishop? Pgs.385-401. In, Bully for Brontosaurus. Reflections in Natural History. W.W. Norton & Company. N.Y., N.Y.

Keynes R- 2003-Fossils, Finches and Fuegians. Darwin's adventures and discoveries on the Beagle. 428 pgs. Oxford University Press. Oxford, Great Britian.

King-Hele D- 1999-Erasmus Darwin. A life of unequaled achievement. 422 pgs. Giles de la Mare Publishers Ltd. London, UK.

Repcheck J- 2003-The man who found time. James Hutton and the discovery of the Earth's antiquity. 247 pages. Perseus Pub., Cambridge, MA

Slotten R.A- 2004-The heretic in Darwin's Court: The Life of Alfred Russel Wallace 602 pages. Columbia University Press, N.Y.

Stott R- 2003-Darwin and the Barnacle. 309 pages. W.W. Norton & Company. N.Y., N.Y.

Related issues:

Darwin published many articles and texts. For a bit more on **Charles Darwin's work** and descriptions of it see:

Darwin C- 1859-<u>The origin of species by means of natural selection or the preservation of favoured races in the struggle for life</u>. 477 pgs. Penguin books. London, U.K.

Darwin C- 1864-On the sexual relations of the three forms of *Lythrum salicaria*- Proceedings of the Linnean Society 8: 169-196

Darwin C- 1881-<u>The Power of Movement in Plants</u>- D. Appleton and Co. N.Y., N.Y. 592 pgs. (1996 reprint, by DeCapo Press.)

Gould S.J- 2002-The essence of Darwinism and the basis of modern orthodoxy: An exegesis of the *Origin of Species*- Chapter 2, pgs. 93-169, in, <u>The structure of evolutionary theory</u>. Belknop Press. Cambridge, MA.

Mayr E- 2000-Darwin's influence on modern thought- Scientific American 283: (#1) 78-83

Mayr E- 2001-<u>What evolution is</u>- 318 pgs. Basic Books. N.Y., N.Y.

Stott R- 2003-<u>Darwin and the Barnacle</u>. 309 pgs. W.W. Norton & Co. N.Y., N.Y.

There is support for **natural selection** at many levels. In addition to classical selection for phenotypic traits, including **sexual selection**, we can now study **molecular selection** at the level of the DNA sequence.

Barrett R.D.H., S.M. Rogers, D. Schluter- 2008-Natural selection on a major armor gene in three spine stickleback- Science 322: (#5899, 10/10) 255-257

Boag P.T., P.R. Grant- 1981-Intense natural selection in a population of Darwin's finches (Geospizinae) in the Galápagos- Science 214: (#4516, 10/2) 82-85

Clutton-Brock T- 2007-Sexual selection in males and females- Science 318: (#5858, 12/21) 1882-1885

Orr A- 2009-Testing natural selection- Scientific American 300: (#1, Jan) 44-50

For more on the **previous work** done by others that related to evolutionary models see:

Eiseley L- 1958-<u>Darwin's century: Evolution and the men who discovered it</u>- 378 pgs. Anchor Books. N.Y., N.Y.

Gould S.J- 2002-Internalism and laws of form: Pre-Darwinian alternatives to functionalism- Chapter 4, pgs. 251-341, in, <u>The structure of evolutionary theory</u>. Belknop Press. Cambridge, MA.

Repcheck J- 2003-<u>The man who found time. James Hutton and the discovery of the Earth's antiquity</u>. 247 pages. Perseus Pub., Cambridge, MA.

Here are a few works that give more on **Charles Darwin** and on some of the people of his time. Including: A biography of Charles Darwin written by his son **Francis Darwin**. **Fitzroy**, the captain of the Beagle.   **Erasmus Darwin**, Charles Darwin's grandfather who wrote many comic poems touching on nature and speciation. An article on work of Darwin's botanist friend, **Joseph Hooker**, concerning early efforts to make biology a distinct profession. And an excellant biography of the much ignored co-discoverer of evolution: **Alfred Russel Wallace**.

Bowler P.J- 2009-Darwin's originality- Science 323: (#5911, 1/9) 223-226

Browne J- 1995-<u>Charles Darwin: Voyaging</u>-606 pgs. Princeton University Press. Princeton, N.J.

Darwin F- 1899-The botanical work of Darwin- Annals of Botany 13: ix-xix

Desmond A., J. Moore- 1991-<u>Darwin: The life of a tormented evolutionist</u>- 808 pgs. Warner Books. N.Y., N.Y.

Endersby J- 2009-Lumpers and splitters: Darwin, Hooker,and the search for order- Science 326: (#5959, 12/11) 1496-1499

Gopnik A- 2006-Rewriting nature- The New Yorker, Oct. 23, pgs. 52-59

Gribbin J., M. Gribbin- 2003-<u>FitzRoy: The remarkable story of Darwin's captain and the invention of the weather forecast</u>. Yale University Press. New Haven, Conn. 336 pgs.

Keynes R- <u>2003-Fossils, Finches and Fuegians. Darwin's adventures and discoveries on the Beagle</u>. 428 pgs. Oxford University Press. Oxford, Great Britian.

King-Hele D- 1999-<u>Erasmus Darwin. A life of unequaled achievement</u>. 422 pgs. Giles de la Mare Publishers Ltd. London, UK.

King-Hele D-1989-Chronicle of the lustful plants: A comic scientific poem written by a doctor-inventor, and published 200 years ago, created a sensation in the literary world- New Scientist April 22, 122: 57-61

Kingsley D.M- 2009-From atoms to traits- Scientific American 300: (#1, Jan) 52-59

Slotten R.A- 2004-<u>The heretic in Darwin's Court: The Life of Alfred Russel Wallace</u> 602 pages. Columbia University Press, N.Y.

Stix G- 2009-Darwin's living legacy- Scientific American 300: (#1, Jan) 38-43

What can be involved in being a biologist and defending **Darwinian evolution**? Here is an article by one of the biologists who testified at the **Scopes trial** as an expert on evolution.

Cole F.C- 1959-A witness at the Scopes trial- Scientific American 200: (#1) 120-130

There has been some back and forth in terms of **acceptance of evolution** by religion and religious leaders lately. The text by Gould is one of the finest arguments I have found on the line between the roles of science and religion in our lives.

Couzin J- 2008-Evolution: Crossing the divide- Science 319: (#5866, 2/22) 1034-1036
Gould S.J- 1999-<u>Rock of Ages: Science and religion in the fullness of life</u>- 241 pgs. The Library of Contemporary Thought. N.Y., N.Y.
Holden C- 2005-Vatican astronomer rebuts cardinal's attack on Darwinism- Science 309: (8/12) 996-997

The latest version of "creationism" is called "**intelligent design**." Behe is one proponent of this view. For those who are interested here is the citation of his text, and how some biologists respond to these views.

Behe M.J- 1996-<u>Darwin's black box: The biochemical challenge to evolution</u>- 307 pgs. The Free Press. N.Y., N.Y.
Brumfiel G- 2005-Who has designs on your student's minds?- Nature 434: 1062-1065
Rennie J- 2002-15 answers to creationist nonsense- Scientific American 287: (#1) 78-85

And for those who wish to cover Darwin and his work in more of a **graphic novel** form.....

Miller J., B. Van Loon- 1982-<u>Darwin for beginners</u>. 176 pgs. Pantheon Books. N.Y., N.Y.

BIO 108   2010
Day 10, Lecture 26, Title: Invertebrates.
Text Readings: Campbell et al. (2008), Chapters 32 and 33.

Topics to cover:

    Animals
    Who are invertebrates?
    Groups we will focus upon
    Traits and functions
    Sponges
    Hydra
    Planaria
    Earthworms
    Clams
    Squids
    Arthropods
Draw tree on board:
    Choanoflagellate
    Animals
        "Porifera"   Sponges (Silicea and Calcarea)
        Eumetazoa
            Radiata   Hydra (Cnidaria)
            Bilateria
                Deuterostomes   Humans (Chordata)
                Protostomes
                    Lophotrochozoa   Planaria (Platyhelminthes)
                                          Earthworms (Annelida)
                                        Clams and Squid (Mollusca)
                Ecdysozoa   Insects (Arthropoda)
Will cover animals groups in this tree, and note some of their traits.

Animals
    Kingdom Animalia
    An outgroup for animals might be a protistian choanoflagate.
        Fig. 32.3, Choanoflagellate
        A multicellular heterotrophic eukaryote, has diploid multicellular stage.
        Sperm with single flagella.
    Some shared derived traits of animal clade...
        Fig. 32.2, AnimalOriginHypoth
        Have interrupted meiosis in formation of egg.
        Development through at least a blastula stage.
            Many go on to do gastrulation, but not all...

Who are invertebrates?

Invertebrates,

      Fig. 32.11, AnimalPhylSSUrRNA

      Invertebrate group is NOT a clade, it is a paraphyletic group..

            All animals share a common ancestor,

            but invertebrate group does not share all descendants from one ancestor

      Those spineless animals completely lacking in vertebrae.

      Want to survey several living groups, note their characteristics, and how they deal with

            several functional needs.

      Good news: phyla are fairly stable (though may be more yet to be discovered...)

      Bad news: how the phyla are related is not set...

            Fig. 32.12, (Campbell and Reece, 2002) MoledVsBodyPlan

            We will use the newer relations, but it will likely shift over time.

Groups we will focus upon

      Fig. 32.11, Animal PhylSSUrRNA

      Sponges   ("Porifera" now includes several phyla, not a monophyletic group)

      Hydra   (Phylum Cnidaria)

      Planaria   (Phylum Platyhelminthes)

      Earthworms   (Phylum Annelida)

      Molluscs   (Phylum Mollusca)

          Clams (Class Bivalvia)

          Squids (Class Cephalopoda)

      Arthropods   (Phylum Arthropoda)

We will try to note how these phyla are related to each other,

         and to identify shared derived traits.

Do not fall into the trap of thinking that just because these groups are not us that they are

         inferior. Species in these groups are successful, and many have lasted far longer

         than humans.

Traits and functions

      General Functions: Some processes that all animals must handle well to survive.

      Animals small in size can have diffusion account for many of these functional needs

         as size becomes larger, or need for exchange increase, then need distinct systems...

         gas exchange

            aerobic life style gives more energy, needed for active life style

               so if sessile, then less need...

            need to take up O_2 and release CO_2

            can do this by being thin, small

               OR can have a circulatory system of some type

            surface area issues come up either way

waste excretion

what type of nitrogenous waste to excrete

aquatic vs. terrestrial living imposes different demands here

water loss issues, and toxicity of waste forms

mechanical support

easiest if in ocean and buoyant

can involve exoskeletons, hydrostatic skeletons, endoskeletons

internal circulation

good for gas exchange, food movement, etc

essential if large, or highly active life style

movement

could be sessile, or sedentary

but if predatory then there tends to be selection for movement.

General Traits

body symmetry

Fig. 32.7, BodySymmetry

types of body symmetry include: none, radial, bilateral

body cavities

Fig. 32.8, Bilateria BodyPlans

What is a body cavity? Coelom. Recall need for tight junctions...

An internal body space with NO opening to the outside

Examples: abdominal cavity, pericardial cavity, etc.

Things that are NOT coeloms (as have openings to the outside):

digestive tract, urogenital tract, respiratory chambers

protostomes vs. deuterostome ensemble of traits

Fig. 32.9, ProtoDeuterostDev

protostomes: "first the mouth" mouth develops first

annus develops second, so complete digestive tract

deuterostomes: "second the mouth" mouth develops second

annus develops first, also a complete digestive tract

Other traits associated with protostomes:

early spiral and determinate cleavage patterns

Patterns of coelom development differ

larval types

Fig. 32.13, TrochophoreLarva

trochophore larva, seen in earthworms and molluscs

ecdysis

Fig. 32.12, Ecdysis

the molting of an exoskeleton, typically containing chitin

see this done in insects and nematodes

Sponges ("Porifera")

Fig. 33.2, (Campbell and Reece, 2002)SpongesCollage

"Porifera" is likely several phyla, so paraphyletic, also sometimes called "Parazoa"

Has the common animal traits; develop through blastula, interrupted meiosis... etc...

No body symmetry at all: not radial, not bilateral

Filter feeders that trap food and do phagocytosis. Note the Chanocytes.

Fig. 33.4, SpongeAnatomy

so eat things smaller than their cells. Do intracellular digestion.

flagella move water in one direction

Sessile, and thin

so gas and waste exchange easy to achieve, no specialized structures needed

Issue of tissues...

Tissue: "An integrated group of cells with a common function, structure, or both."
(by glossary; Campbell et al. 2008)

But sponges said to not have "true" tissues? (See pg. 659; Campbell et al., 2008)
"True tissues are collections of specialized cells isolated from other
tissues by membranous layers." This extracellular matrix structure is
what separates the parazoa from the eumetazoa, has to do with "truth"

Fig. 33UN-647, (Campbell and Reece, 2002) Parazoa

Note lack of body symmetry, lack of "true" tissues, but otherwise these are animals

Hydra (Phylum Cnidaria)

Others in this group include jellyfish, sea anemones, and corals.

Fig. 33.5, CnidarianForms

polyp and medusa body forms seen, hydra have just polyp form, lack medusa form

Radial body symmetry.

Two "true" tissue layers (i.e. diploblastic): Ectoderm and endoderm. No mesoderm
"mesoglea" is space between the two tissues with some cells,
but no membranous layer, so not a "true" tissue...

Predators, trap food via stinging nematocysts.

Fig. 33.6, HydraCnidocyte

Fig. 29.8, (Purves et al., 1998) nematocyst.jpg

Cnidocytes, have specialized organellles, nematocysts

Some floating, some fairly sessile. May move slowly.

Low metabolic activity and thin body structure

so gas and waste exchange is easily achieved by diffusion alone. No heart...

vascular part of gastrovascular cavity, can close mouth and move water around

Hydrostatic skeleton, uses vascular system in tentacles to extend them...

Gastrovascular cavity, mouth and annus are the same thing,

so no complete digestive tract here...

Quicktime movie; 33.5, HydraEatFlea-B.mov (Campbell and Reece, 2002)

Fig. 33UN-648, (Campbell and Reece, 2002) Radiata

Radial, two tissues, GV cavity, and nematocysts in this phylum...

Planaria (Phylum Platyhelminthes)

Has all three tissue layers (i.e. triploblastic): Ectoderm, mesoderm, endoderm.

Bilateral body symmetry.

    Some cephalization, eye spots at anterior end.

Now in the protostomes proper. But this phylum has lost some ancestral features...

Are Lophotrochozoa. Similar larva type

    Fig. 33UN-651, (Campbell and Reece, 2002) Lophotrochozoa

But planaria have lost complete digestive tract. So this is the loss of a trait.

    Fig. 33.10, PlanarianAnatomy

    Has a gastrovascualar cavity.

        reticulates so can get monomers to cells of body

        Fig. 39.17, (Raven and Johnson, 1995) planaria.jpg

    eats through scrapping food it skims over.

Also has no body cavity. So another trait lost?

Moves via cilia on lower side. A grazer of surface algae.

Thin, slow, so can do gas exchange through skin.

Does have a flame-cell excretory system that does excretion and osmoregulation

Many Platyhelminthes are highly derived parasites (i.e. tapeworms, flukes...)

    so the seemingly simple body plan is a result of specialization,

    it does not represent a true ancestral state

    so these are often lumped with the protostomes,

        even though they lack a complete digestive tract

Earthworms (Phylum Annelida)

Fig. 33.23x, (Campbell and Reece, 2002) EarthwormPhoto

Fig. 46.01, earthworm sex

A more typical protostome. Also a Lophotrochozoan.

    Fig. 33.22, EarthwormAnatomy

    Complete digestive tract. So distinct mouth and anus..

Coeloms present. Each segment of the body has its own body cavity

Metanephridia, for regulating contents of urine produced.

Circulatory system

    aortic arches pump blood to capillary beds close to skin

    it takes a lot of energy to burrow so need good gas exchange

    to promote good gas exchange can manipulate items associated with diffusion

        could have higher surface area, but that would interfere with burrowing?

        could lower metabolic demands, but burrowing is costly

        could make exchange surface thin, but need a tough skin underground

        needs to move blood near body surface quickly,

            this maintains the concentration gradient of oxygen

Body segmentation

    Movie, 33.23, Earthwormlocomotion-B.mov (Campbell and Reece, 2002)

    allows for movement by varying muscle contraction pushing on fluid

    circular and longitudinal muscle actions, which extends? which contracts?

a hydrostatic skeleton

note use of setae (hairs) to anchor itself

Fig. 33UN-651, (Campbell and Reece, 2002) Lophotrochozoa

Earthworm, closed circulation, hydrostatic skeleton, setae, metanephridia

Clams (Phylum Mollusca, Class Bivalvia)

Fig. 33.19, bivalve

Bilateral symmetry. Body cavity.

These have a common body plan: foot, mantle, visceral mass.

Fig. 29.28, (Purves et al., 1998) mollusca.jpg

Fairly sedentary, often filter feeders (though some predatory bivalves exist)

Water flow incurrent siphon and excurrent siphon

Fig. 33.13, (Freeman, 2008) Clam

flow powered by cilia on gills

Gills

Fig. 33.20. ClamAnatomy

for gas exchange and for catching food!

How to catch food? cilia move food particles to base of gills

note flow of water, incurrent and outcurrent siphons play a role

water flow powered by cilia on the cills

food then moved to mouth via labial palp (like a tongue)

Complete digestive tract.

often have a scraping structure, a radula

eat suspended algae and suspended particles

Open circulatory system.

works since they are sedentary

note the digestive tract goes through their heart

so the way to a clam's heart is....

Shell secreted by mantle, gives protection

Foot moves by a muscular/hydrostatic skeleton (like our tongue)

Can use its foot to move, and to burrow

Squids (Phylum Mollusca, Class Cephalopoda)

Fig. 33.21, CephalopodsCollage

These have the same body plan as clams: foot, mantle, visceral mass.

Fig. 29.28, (Purves et al., 1998) mollusca.jpg

Bilateral symmetry. Body cavity is small, but there.

Complete digestive tract, mouth has a radula in beak

annus back in mantle near excurrent siphon, just like clams

eat meat, so a predatory digestive tract, short and simple...

Active predators. Very complex nervous system.
Used water flow to both move and move water over the Gills
> Fig. 33.10, (Freeman, 2008) Squid
> squid.jpg
> Here gill are just gas exchange, not for prey capture as in clams
> Water flow is powered by muscle movement, not cilia as in clams

Closed circulatory system. Good for gas exchange and active life style.
Can become very large!
How flexible are cephalopods?
> octopus and coconut 1 - Steene 1998.jpg
> octopus and coconut 2 - Steene 1998.jpg
> octopus and coconut 3 - Steene 1998.jpg
> octopus and coconut 4 - Steene 1998.jpg

Fig. 33UN-651, (Campbell and Reece, 2002) Lophotrochozoa
> Common body plan: foot, mantle, visceral mass
> Radula

Arthropods

These include crustaceans, millipods, horseshoe crabs, spiders, ticks, insects, etc...
> Fig. 33.35a, (Campbell and Reece, 2002) Lobster
> Fig. 33.30, HorseshoeCrabs
> Fig. 33.30x, (Campbell and Reece, 2002) LycosidSpider
> Fig. 33.31a, (Campbell and Reece, 2002) Millipedes
> Fig. 33.x1, (Campbell and Reece, 2002) Beetle

Fig. 33UN-651-Lophotrochozoa
Now shift from one set of protostomes to another, Lophotrochozoa to Ecdysozoa
Fig. 33UN-661-Ecdysozoa
They have a chitinaceous exoskeleton that they often molt. Ecdysis.
Bilateral symmetry. Coelom present, often with blood in it, a hemocoel.
Lobsters: Have hard exoskeleton
> Fig. 33.29, ArthropodExtAnatom
> Gills under shell, moves water over the gills via action of gill-bailers.
>> these are modified appendages that pull the water forward

Insects:
> Fig. 33.35, InsectAnatomy
> Tracheal system for gas exchange, takes air to cells.
>> Fig. 14.31, (Eckert et al., 1978) insectrachea.jpg
>> diffusion through gaseous phase about 10000 times faster than in liquid
>> this does ultimately limit their size,

Open circulatory system. Not blood, but hemolymph.
     a combination of blood and lymph in one fluid: hemolymph
     since oxygen is not carried in the blood, can be an open system
Waste removal.
     Malphigian tubules.
          Fig. 12.36, (Eckert et al., 1978) malpighian.jpg
          secretion and reabsorption, but no filtration.
     some species use counter current systems to recover water
          Fig. 12.38, (Eckert et al., 1978) countercurrent.jpg
     excretes uric acid, so makes crystals, water conserving
Various means of movement. Exoskeleton and antagonistic muscles.
Perhaps the most successful of animals and highly diverse!

Summary
     Fig. 33.1, (Campbell and Reece, 2002) Animal PhyloReview
     Note where each phylum is grouped into a higher level.
     Also consider how each is adapted for its habit. Different selection pressures...

If Time:
     How to defend yourself against an attack of a giant mutant insect!
          Fig. 33.35, Insect Anatomy
          (How the movies got it wrong...)

Objectives:

What characteristics are typical of all members of the animal kingdom? Know the characteristics of the following groups: "Porifera", Eumetazoa, Bilateria, Lophotrochozoa, Ecdysozoa.

In terms of the traits that are shared, be able to describe the reasoning for assembling phyla into the Lophophozoa and Ecdysozoa clades. What is ecdysis?

Be able to describe several functional issues that all invertebrates must address in order to be successful. What issues must terrestrial invertebrates deal with that are not major problems for marine invertebrates? What are several advantages of being small in size? What are several advantages of being larger in size? What functional issues does each option help address?

Be able to contrast traits typical of protostomes and deuterostomes, and identify examples of animals that belong to each of these groups, and animals that belong to neither of these groups. What characteristics arose in animals long before these groups, and are these groups monophyletic or not? (Looking at figure 32.11 may be helpful here.)

Contrast the characteristics found in the following groups of animals and how they meet the needs of circulation, waste removal, gas exchange, movement and mechanical support: sponges ("Porifera"), hydra (Cnidaria), planaria (Platyhelminthes), earthworms (Annelida), clams and squid (Mollusca), and arthropods (Arthropoda). For these animals know the distinctive traits found in each.

Insects are arthropods that are very successful terrestrial invertebrates. How do insects manage to be such active organisms and yet have an open circulatory system? Being so small, how do they manage to avoid drying out?

What is a coelom, what are examples of it, and what advantage does it give to an organism? What functions can a coelom help to address?

For review, see self-quiz questions #1 and 2 of chapter 32, and questions #1, 2, 3, and you might try doing #7 just for the phyla we have covered, of chapter 33.

Needed overheads and items:

Fig. 32.3, Choanoflagellate
Fig. 32.2, AnimalOriginHypoth
Fig. 32.11, AnimalPhylSSUrRNA
Fig. 32.12, (Campbell and Reece, 2002) MoleVsBodyPlan
Fig. 32.11, Animal PhylSSUrRNA
Fig. 32.7, BodySymmetry
Fig. 32.8, Bilateria BodyPlans
Fig. 32.9, ProtoDeuterostDev
Fig. 32.13, TrochophoreLarva
Fig. 32.12, Ecdysis
Fig. 33.2, (Campbell and Reece, 2002) SpongesCollage
Fig. 33.4, SpongeAnatomy
Fig. 33UN-647, (Campbell and Reece, 2002) Parazoa
Fig. 33.5, CnidarianForms
Fig. 33.6, HydraCnidocyte
Fig. 29.8, (Purves et al., 1998) nematocyst.jpg
Quicktime movie; 33.5, HydraEatFlea-B.mov (Campbell and Reece, 2002)
Fig. 33UN-648, (Campbell and Reece, 2002) Radiata
Fig. 33UN-651, (Campbell and Reece, 2002) Lophotrochozoa
Fig. 33.10, PlanarianAnatomy
Fig. 39.17, (Raven and Johnson, 1995) planaria.jpg
Fig. 46.01, earthworm sex
Fig. 33.23x, (Campbell and Reece, 2002) EarthwormPhoto
Fig. 33.22, EarthwormAnatomy
movie, 33.23, Earthwormlocomotion-B.mov, (Campbell and Reece, 2002)
Fig. 33UN-651, (Campbell and Reece, 2002) Lophotrochozoa
Fig. 33.19, bivalve
Fig. 29.28, (Purves et al., 1998) mollusca.jpg
Fig. 32.23, (Freeman, 2008) Clam
Fig. 33.20. ClamAnatomy
Fig. 33.21, CephalopodsCollage
Fig. 29.28, (Purves et al., 1998) mollusca.jpg
Fig. 33.10, (Freeman, 2008) Squid
squid.jpg
octopus and coconut 1 - Steene 1998.jpg
octopus and coconut 2 - Steene 1998.jpg
octopus and coconut 3 - Steene 1998.jpg
octopus and coconut 4 - Steene 1998.jpg
Fig. 33UN-651, (Campbell and Reece, 2002) Lophotrochozoa
Fig. 33.35a, (Campbell and Reece, 2002) Lobster
Fig. 33.30, HorseshoeCrabs
Fig. 33.30x, (Campbell and Reece, 2002) LycosidSpider
Fig. 33.31a, (Campbell and Reece, 2002) Millipedes

Fig. 33.x1, (Campbell and Reece, 2002) Beetle
Fig. 33UN-651, (Campbell and Reece, 2002) Lophotrochozoa
Fig. 33UN-661, (Campbell and Reece, 2002) Ecdysozoa
Fig. 33.29, ArthropodExtAnatom
Fig. 33.35, InsectAnatomy
Fig. 14.31, (Eckert et al., 1978) insectrachea.jpg
Fig. 12.36, (Eckert et al., 1978) malpighian.jpg
Fig. 12.38, (Eckert et al., 1978) countercurrent.jpg
Fig. 33.1, (Campbell and Reece, 2002) Animal PhyloReview
Fig. 33.35, InsectAnatomy

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-An introduction to animal diversity. Chapter 32. Pages 654-665. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Invertebrates. Chapter 33. Pages 666-697. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-Biology, 6th edition, Figures 32.12, 33.1, 33.2, 33.23x, 33.35a, 33.30x, 33.31a, 33.x1, 33UN-647, 33UN-648, 33UN-651; Movies 33.5, 33.23. Benjamin Cummings Press. San Francisco, CA.

Eckert R., D. Randall- 1978-Osmoregulation and excretion- Figures 12.36, 12.38, 14.31. In Animal Physiology. W.H. Freeman and Company, San Francisco

Freeman S- 2008-Biological Science. Third edition. Figures 33.10 and 33.13. Pearson Education Inc. San Francisco, CA.

Purves W.K., G.H. Orians, H.C. Heller, D. Sadava- 1998-Life. The Science of Biology. 5th edition. Figures 29.8, 29.28. Sinauer Associates, Inc. Sunderland, MA.

Raven P.H., G.B. Johnson- 1995-Biology, 3rd edition. Figure. 39.17. Wm. C. Brown Publishers. Dubuque, Iowa.

Steene R- 1998-Coral Seas. 272 pgs. Firefly Books Inc. Buffalo N.Y.

Related issues:

For some good reviews of **invertebrate diversity** and aspects of their **physiology** see:

> Eckert R., D. Randall- 1978-<u>Animal Physiology</u>. 558 pgs. W.H. Freeman and Company, San Francisco
> Ruppert E.E., R.D. Barnes- 1994-<u>Invertebrate Zoology</u>. 1056 pages. Saunders College Publishing, Fort Worth.

Here are a few recent articles about **squids**. Their beaks are reported to be one of the hardest biological materials ever found.

> Messersmith P.B- 2008-Multitasking in tissues and materials- Science 319: (#5871, 3/28) 1767-1768
> Miserez A., T. Schneberk, C. Sun, F.W. Zok, J.H. Waite- 2008-The transition from stiff to compliant materials in squid beak- Science 319: (#5871, 3/28) 1816-1819

Echinoderms are invertebrates who have many interesting traits. The materials in the sea cucumber has inspired biomimicing materials.

> Capadona J.R., K. Shanmuganathan, D.J. Tylor, S.J. Rowan, C. Weder- 2008-Stimuli-responsive polymer nanocomposites inspired by the sea cucumber dermis- Science 319: (#5868, 3/7) 1370-1374

Studies of the **platyhelminthes** include the abilities of planaria to carry out wound regeneration. This might tell us something about how our own wound recovery can be enhanced?

> Gurley K.A., J.C. Rink, A.S. Alvarado- 2008-β-catenin defines head versus tail identity during planarian regeneration and homeostasis- Science 319: (#5861, 1/18) 323-327
> Petersen C.P., P.W. Reddien- 2008-Smed-β-catenin-1 is required for anteroposterior blastema polarity in planarian regeneration- Science 319: (#5861, 1/18) 327-330

Cnidarians include species that make up corals.

> Jones C- 2007-Moonlight sonata on the reef- Science 318: (#5857, 12/14) 1715
> Levy O., L. Appelbaum, W. Leggat, Y. Gothitf, D.C. Hayword, D.J. Miller, O. Hoegh-Guldberg- 2007-Light-responsive cryptochromes from a simple multicellular animal, the coral *Acropora millepora*- Science 318: (#5849, 10/19) 467-470
> Pennisi E- 2007-Spawing for a better life- Science 318: (#585, 12/14) 1712-1717

Studies of insects and other **arthropods** are ongoing. Including the **social insects**, such as bees, and the basis for the determination of their queens. How their **larval stages** are used to hide from or deter predators. There is also much interest in what is causing bees to go through colony collapses.

Cox-Foster D.L., S. Coulan, E.C. Holmes, G. Palacios, J.D. Evans, N.A. Moran, P-L. Quan, T Briese, M. Hornig, D.M. Geiser, V. Martinson, D. van Engelsdorp, A.L. Kalkstein, A. Drysdale, J. Hui, J. Zhai, L. Cui, S.K. Hutchison, J.F. Simons, M. Egholm, J.S. Pettis, W.I. Lipkin- 2007-A metagenomic survey of microbes in honey bee colony collapse disorder- Science 318: (#5848, 10/12) 283-287

Futahashi R., H. Fujiwara- 2008-Juvenile hormone regulates butterfly larval pattern switches- Science 319: (#5866, 2/22) 1061

Kucharski R., J. Maleszka, S. Faret, R. Maleszka- 2008-Nutritional control of reproductive status in honeybees via DNA methylation- Science 319: (#587, 3/28) 1827-1830

Pennisi E- 2007-Fruit fly blitz shows the power of comparative genomics- Science 318: (#5852, 11/9) 903

Stokstad E- 2007-Puzzling decline of U.S. bees linked to virus from Australia- Science 317: (#5843, 9/7) 1304-1305

Toth A.L., K. Varala, T.C. Newman, F.E. Miguez, S.K. Hutchison, D.A. Willoughby, J.F. Simons, M. Egholm, J.H. Hunt, M.E. Hudson, G.E. Robinson- 2007-Wasp gene expression supports an evolutionary link between maternal behavior and eusociality- Science 318: (#5849, 10/19) 441-444

Vergoz V., H.A. Schreurs, A.R. Mercer- 2007-Queen pheromone blocks aversive learning in young worker bees- Science 317: (#5836, 7/20) 384-386

Whitfield J- 2007-Who's the queen? Ask the genes- Science 318: (#5852, 11/9) 910-911

Many **sponges** have been found to have bacterial **endosymbionts** in many of their cells. Also, unlike other animals, many sponges have **collagen** that lacks certain covalent modifications.

Vanacore R., A-J.L. Ham, M. Voehler, C.R. Sanders, T.P. Conrads, T.D. Veenstra, K.B. Sharpless, P.E. Dawson, B.G. Hudson- 2009-A sulfilimine bond identified in collagen IV- Science 325: (#5945, 9/4) 1230-1234

Vogel G- 2008-The inner lives of sponges- Science 320: (#5879, 5/23) 1028-1030

BIO 108   2010
Day 11, Lecture 27, Title: Bioassays.
Text Readings: Campbell et al. (2008), pgs. 583-584 (malaria), 798 (dodder), 821-841.
Article Readings: (PDF versions posted on course web site.)
        Runyon J.B., M.C. Mescher, C.M. DeMoraes- 2006-Volatile chemical cues guide host
                location and host selection by parasite plant- Science 313: (9/29) 1964-1967.
        Lacroix R., W.R. Mukabana, L.C. Gouagna, J.C. Koella- 2005-Malaria infection
                increases attractiveness of humans to mosquitoes- Public Library of Science
                Biology 3: (#9) e298, 4 pages.

Topics to cover:

        What is a Bioassay and what are its uses?
        Growth assays
                Plant hormone detection
                Quantitative uses of bioassays
        Other examples
                Ames Test
                Malarial strategy to attract Mosquitoes
                Parasitic plant detection of prey
        Summary

What is a Bioassay and what are its uses?
Definition of a bioassay
        Use of a biological system to detect the presence of some item or signal.
                We detect it by the response of the biological system,
                        responses can be growth, behavioral, etc....
This makes use of an organism's ability to detect the item, so we are dealing with receptors
        Fig. 39.3, signal-transduction systems
        Specific receptor, typically a protein but not always
        specific ligand we are trying to detect, typically a small organic molecule,
                but can be inorganic, we need not know what it is at first...
        Useful for identification of active factor in extracts
                crude extracts have many items
                which are important for action?
        Example, greening response in plants
                Fig. 39.2, potato greening
                what receives the signal, and how is response turned on?
                Phytochrome is the light receiving molecule
                        Fig. 39.19, phytochrome
                So can use this as a bioassay for certain treatments of light
                        Fig. 39.4, signal transduction, detailed...

1

When have knowledge of system can go into the parts of it, how does it work?
    How important is kinase activity?
    Is calcium ion concentration important?
    Is gene expression involved in response or not?
    So a bioassay can give us information about
        the signal, transduction, response systems as well
This is critical for pathogen studies
    Does the virus target one cell type? Do a bioassay
    Is the pathogen able to block immune system action? Do a bioassay
    Is the growth of the pathogen slowed by a drug? Do a bioassay

Growth assays

In some cases the response is growth, change in growth rate or growth pattern
Consider how this is different from giving more food or nutrients...

Plant hormone detection

Growth assays: Auxin
    Auxin is a plant hormone, detected by a bioassay system
    Fig. 39.6, Went experiments, example of bioassay
    Collected exuded items.
        Not pure. But a starting point.
    Use of coleoptile curving as biological response.
    Can then purify and fractionate the items in the extract and use bioassay again
        this allowed the identification of the active agent, in this case auxin
Growth assays: Gibberrellin
    Similar case used to identify this other plant hormone
    Examples of "foolish rice" diseases and dwarf peas
        Fig. 39.9, (Campbell and Reece, 2002) foolish rice
        Fig. 39.10, (Campbell and Reece, 2002) dwarf peas
        we select for shorter plants, as grow faster, use less fertilizer, etc...
        To be short, often have a block in their biochemical pathway for hormone
            production.
        So if we supply the hormone, the plant reponds, and grows taller
    Some fungi use this to attack plants, longer spindly plants are easier for fungus to
        attack. So a fungus was found that made GA and secreted it onto plants

Quantitative uses of bioassays

Qualitative bioassays can detect presence or not.

Quantification gives an estimate of concentration of an item.

In some cases, can use a bioassay to quantify concentration of item in an extract.

> bioassay.tif

But to do this the response has to display a clear linear response range.

Below threshold, no significant response seen

> Receptor has a Km for item, what if below Km? No response.
>
> Why else might there be a threshold concentration?
>
> > Item of interest is likely food for bacteria, is the system sterile?
> >
> > Item of interest is large
> >
> > > what if receptor is in cell? can it cross membrane? Is it degraded?

Linear response, more item added, gives more response

> in this range the amount of response
>
> > can be related unambiguously to the concentration of the item.
>
> So this is a standard curve relationship between the bioassay system response
>
> > and the concentration of active agents in the extracts applied

Saturation and beyond, what if all of receptors are saturated already? Then no further response.

> What if item is toxic at high doses?   response increases but death also occurs?

Consider the relative concentrations involved here

> often a plant growth hormone is used at concentrations of 1×10^{-6} Molar.
>
> > This is much lower than the concentration of sugars, amino acids, etc...
> >
> > So can not be a source of organic carbon, not a food or mineral source

Other examples

> **Ames Test**

This test is used to detect mutagens

> Fig. 9.81, (Madigan etal, 1997) Ames_test.jpg

plate bacterium evenly

> This bacterium is a mutant strain
>
> > has a block for Histidine synthesis (HIS: an essential amino acid)
> >
> > this block is due to one point mutation in the biosynthetic pathway for HIS
> >
> > > so must have HIS to live
>
> Put evenly on medium lacking HIS
>
> > so only if back mutations occur in the mutated gene will it live and grow
>
> Expose the plated bacteria to various concentrations of suspected mutagen
>
> > look for enhanced rates of back mutations
>
> This is used to evaluate mutagenesis, which is correlated to cancer-induction
>
> > mutagenic items tend to be carcinogenic ones
>
> this is one way to check if food coloring is safe to put in human food...

Malarial strategy to attract Mosquitoes

Plasmodium is a protistian parasite, in us it causes malaria

> Fig. 28.10, Plasmodium life cycle
>
> To be a successful parasite it must achieve different things at different stages
>
> When a gametocyte it must get out of human and into insects
>
>> So does the presence of gametocytes in human attract mosquitoes?
>
> Study by Lacroix et al. (2005)

Experimental set up

> Fig. 1. (Lacroix et al., 2005)
>
> Note use of three people at a time.
>
>> one uninfected
>>
>> one infected but the parasites are not making gametocytes yet
>>
>> one infected and the parasites are at gametocyte stage
>
> Mosquitoes exposed to odors from these three people
>
>> Experiment repeated after all three were treated to remove infection
>>
>>> How is this a good control? What else contributes to human odors?

Results and Conclusions

> Fig. 2. (Lacroix et al., 2005)
>
> Note that only presence of gametocytes led to higher mosquito attraction.

So this bioassay can then be used to try to determine what compound acts as the odor

> can then try to see how the parasite induces its production and release in humans...
>
> can also try to determine how the mosquitoes detect this compound...

Parasitic plant detection of prey

Dodder is a parasitic plant. Feeds by connecting to host plant's vascular tissue

> gets water, minerals, and organic matter from host plant
>
>> Fig. 1. (Runyon et al., 2006)
>
> A problem for some agricultural crops.
>
> Some host plants feed it better than others. Can dodder detect different potential hosts?
>
> Study by Runyon et al. (2006)

Experimental setup

> Fig. 2. (Runyon et al., 2006)
>
> Two types of experiments
>
>> 1.) expose dodder to two-sided options with combinations of:
>>
>> moist soil, tomato plant, extracts from tomato
>>
>> 2.) expose dodder to four sided options with combinations of:
>>
>> extracted and partially purified extracts from plants

Results and Conclusions

> Tomato attracts dodder, wheat repells
>
>> Table 1, (Runyon et al., 2006)
>>
>> so must sense something given off by these plant?

Extracts from plants tested, and some found to attract dodder, others to repell
Table 4, (Runyon et al., 2006)
This allows a study of the senses of dodder
if it can be attracted or repelled by different volatile compounds
then what does that suggest about the number of receptors it has?
if the receptors that detect repellant compounds can be activated this might
provide some protection to farm plants?

Summary
Any response by a biological system to a stimulus can be used as a bioassay.
these studies can be done for a wide variety of reasons.
can allow identification of the signaling molecule(s) involved
these can be pheromones, hormones, etc...
Or depending on interests this can get to the molecular level of the response systems
Fig. 39.4, signal transduction in light response
allowing study of
receptors, signal transduction, responses.... of biological interest.
This includes such things as:
what signals do neurons detect when they migrate early on in development?
could pheromones be used to trap insect pests?

Objectives:

Growth assays and the Ames test are examples of bioassays. How specific is each of these bioassays? Is specificity always desired? Can you think of examples of when it might be an advantage to have a broader test? Be able to describe how the Ames test operates, and what it is designed to detect. From your reading, be able to identify several plant responses that have been studied by the use of bioassays. Be able to describe how a growth response to a hormone is different from a growth response due to the supply of more nutrients.

The evaluation of threshold, linear response, and saturation levels of response are critical aspects of quantitative bioassay systems. What can cause a treatment to result in a response that is below threshold, or to its being above the saturation level? Be able to describe the state of the receptor when at any of these three levels of response.

Be able to describe the studies done by Lacroix et al. (2005) and Runyon et al. (2006) in terms of the experimental questions being addressed, the methods used, and the important results and conclusions reached. In each case what aspects of the experimental design helped to reduce the effects of extraneous variation on the results? In each of these studies what would be a next logical step to take?

For review, see self-quiz question #1 of chapter 39.

Needed overheads and items:

Fig. 39.3, signal-transduction systems
Fig. 39.2, potato greening response
Fig. 39.19, phytochrome
Fig. 39.4, signal transduction, detailed...
Fig. 39.6, Went experiments, example of bioassay
Fig. 39.9, (Campbell and Reece, 2002) foolish rice
Fig. 39.10, (Campbell and Reece, 2002) dwarf peas
bioassay.tif
Fig. 9.8, (Madigan etal, 1997) Ames_test.jpg
Fig. 28.10, plasmodium life cycle
Fig. 1, (Lacroix et al., 2005)
Fig. 2, (Lacroix et al., 2005)
Fig. 1, (Runyon et al., 2006)
Fig. 2, (Runyon et al., 2006)
Table 1, (Runyon et al., 2006)
Table 4, (Runyon et al., 2006)
Fig. 39.4, signal transduction

Readings: (post as PDF files on website)
Runyon et al. (2006)
Lacroix et al. (2005)

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky,
R.B.Jackson- 2008-<u>Biology</u>. Eighth edition. Pgs. 583-584, 798, 821-841.
Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figures 39.9, 39.10.
Benjamin Cummings Press. San Francisco, CA.

Lacroix R., W.R. Mukabana, L.C. Gouagna, J.C. Koella- 2005-Malaria infection
increases attractiveness of humans to mosquitoes- Public Library of Science
Biology 3: (#9) e298, 4 pages.

Madigan M.T., J.M. Martinko, J. Parker- 1997-<u>Biology of Microorganisms</u>. 8th edition.
Figure 9.8. Prentice Hall Inc. Upper Saddle River, N.J.

Runyon J.B., M.C. Mescher, C.M. DeMoraes- 2006-Volatile chemical cues guide host
location and host selection by parasite plant- Science 313: (9/29) 1964-1967

Related issues:

Bioassays are used for drug screening and **environmental safety** screenings.

> Collins F.S., G.M. Gray, J.R. Bucher- 2008-Transforming environmental health
> protection- Science 319: (#5865, 2/15) 906-907

Drug development often makes use of bioassays to assess a candidate drug's affects on gene expression.

> Lamb J., E.D. Crawford, D. Pack, J.W. Modell, I.C. Blat, M.J. Wrobel, J. Lemer, J-P.
> Brunet, A. Subramanian, K.N. Ross, M. Reich, H. Hieronymus, G. Wei, S.A.
> Armstrong, S.J. Haggarty, P.A. Clemans, R. Wei, S.A. Carr, E.S. Lander, T.R.
> Galub- 2006-The connectivity map: Using gene-expression signatures to connect
> small molecules, genes, and disease- Science 313: (9/29) 1929-1935

This study describes the use of **fish responses** as a bioassay system for **screening for new drugs**.

> Rihel J., D.A. Prober, A. Arvanites, K. Lam, S. Zimmerman, S. Jang, S.J. Haggarty, D.
> Kokel, L.L. Rubin, R.T. Peterson, A.F. Schier- 2010-Zebrafish behavioral profiling
> links drugs to biological targets and rest/wake regulation- Science 327: (#5963,
> 1/15) 348-351

These two studies describe a bioassay for **toxins** in a common plant in our area which if eaten by cattle can kill them, and which can get into cow milk and has killed humans.

> Beier R.C., J.O. Norman, T.R. Irvin, D.A. Witzel- 1987-Microsomal activation of
> constitutents of white snakeroot (*Eupatorium rugosum* Hoatt) to form toxic
> products- American Journal of Verterinary Research 48: (#4) 583-589
> Kaufmann G.W- 1982-Seasonal variation of tremetol concentrations found in white snake
> root, *Eupatorium rugosum* Houtt (Compositae)- Proceedings of the Iowa
> Academy of Science 89: (#4) 151-152

This bioassay used the shift of invertebrate larvae to asexual reproduction as a means to identify **chemical signals** from predators that these **echinoderms** sense.

> Vaughn D., R.R. Strathmann- 2008-Predators induce cloning in echinoderm larvae-
> Science 319: (#5869, 3/14) 1503

This bioassay examines **mosquito** responses to odors from a plant.

Healy T.P., P.C. Jepson- 1988-The location of floral nectar sources by mosquitoes: the long-range responses of *Anopheles arabiensis* Patton (Diptera: Culicidae) to *Achillea millefolium* flowers and isolated floral odour- Bulletin of Entomological Research 78: 651-657

This study uses a bioassay system to try to determine which **self-antigens** our immune system might be able to recognize. A topic of importance if the immune system is to attack tumor cells.

Savage P.A., K. Vasseller, C. Kang, K. Larimore, E. Riedel, K. Wojnooski, A.A. Jungbluth, J.P. Allison- 2008-Recognition of a ubiquitous self antigen by prostate cancer-infiltrating CD8$^+$ T lymphocytes- Science 319: (#5860, 1/11) 215-220

This bioassay uses the growth response of corn seedlings to various fungi isolated from soil to determine which might be involved in **mycorrhizeal** interactions with plants.

Anderson R.C., E.S. Menges- 1997-Effects of fire on sandhill herbs: Nutrients, mycorrhizae, and biomass allocation- American Journal of Botany 84: (#7) 938-948

This paper presents the use of a bioassay to study the influence items bound by **cannabinoid receptors** on neuron growth. (Hmmm.... Wonder where they got the cannabinoids... ;-)

Berghuis P., A.M. Rajnicek, Y.M. Morozov, R.A. Ross, J. Mulder, G.M. Urbán, K. Monory, G. Marsicano, M. Matteoli, A. Canty, A.J. Irving, I. Katona, Y. Yanagawa, P. Rakic, B. Lutz, K. Mackie, T. Harkany- 2007-Hardwiring the brain: Endocannabinoids shape neuronal connectivity- Science 316: (5/25) 1212-1216

For a bit more about the **dodder** bioassay system see:

Pennisi E- 2006-Parasitic weed uses chemical to find host plant- Science 313: (9/29) 1867

Prions are infectious misfolded proteins. These articles describe a bioassay system to study **prions** and attempts to find other cofactors that prions might use to promote their infectivity.

Supattapone S- 2010-What makes a prion infectious?- Science 327: (#5969, 2/26) 1091-1092
Wang F., X. Wang, C-G. Yuan, J. Ma- 2010-Generating a prion with bacterially expressed recombinant prion protein- Science 327: (#5969, 2/26) 1132-1135

Here are some examples of the uses of bioassay systems to identify new **plant hormones**.

Altmann T- 1999-Molecular physiology of brassinosteroids revealed by the analysis of mutants- Planta 208: 1-11

Aspinall D., L.G. Paleg, F.T. Addicott- 1967-Abscisin II and some hormone-regulated plant responses- Australian Journal of Biological Sciences 20: 869-882

Christianson M.L., J.S. Hornbuckle- 1999-Phenylurea cytokinins assayed for induction of shoot buds in the moss *Funaria hygrometrica*- American Journal of Botany 86: (#11) 1645-1648

Mitchell J.W., D.P. Skaggs, W.P. Anderson- 1951-Plant growth-stimulating hormones in immature bean seeds- Science 114: (#2954) 159-161

Here are some articles that deal with the **malarial parasite** and attempts to control it.

Dvorin J.D., D.C. Martyn, S.D. Patel, J.S. Grimley, C.R. Collins, C.S. Hopp, A.T. Bright, S. Westenberger, E. Winzeler, M.J. Blackman, D.A. Baker, T.J. Wandless, M.T. Duraisingh- 2010-A plant-like kinase in *Plasmodium falciparum* regulates parasite egress from erythrocytes- Science 328: (#5980, 5/14) 910-912

Kappe S.H.I., A.M. Vaughan, J.A. Boddey, A.F. Cowman- 2010-That was then but this is now: Malaria research in the time of an eradication agenda- Science 328: (#5980, 5/14) 862-866

Kumar S., A. Molina-Cruz, L. Gupta, J. Rodrigues, C. Barillas-Mury- 2010-A peroxidase/dual oxidase system modulates midgut epithelial immunity in *Anopheles gambiae*- Science 327: (#5973, 3/26) 1644-1648

Milhous W.K., P.J. Weina- 2010-The botanical solution for malaria- Science 327: (#5963, 1/15) 279-280

BIO 108   2010
Day 12, Lecture 28, Title: Post-Darwinian Evolution.
Text Readings: Campbell et al. (2008) Chapter 24, and pgs. 483-484, 525-531.

Topics to cover:

> **What is a species?**
> **Things that separate species?**
> **Allopatric speciation**
> **Darwinian evolution**
> **New additions to the model....**
> > **sympatric speciation**
> > **punctuated equilibrium**
> > **Rapid changes by change in developmental tempo**
> > **Other levels of selection?**

What makes a species a species? The answer is not clear-cut, but depends on the context. The model of evolution has been modified greatly from what Darwin originally proposed. We will consider ways in which speciation may occur, first as Darwin envisioned the process, and then by new mechanisms. The finding of new means for speciation to occur has expanded the model of evolution, but it is likely that it is still not complete.

What is a species?
> Will focus mainly on eukaryotic species, they come in various contexts...
> with just a mention of Prokaryotic concepts of "species."
> > biological species
> > > ability to mate and produce viable offspring, a reproductive test
> > > Fig. 39.8, mating attempts (Purves etal. 1998)
> > > A tough standard to meet?
> > Morphological/Taxonomic species
> > > Fig. 25.4, fossils
> > > fossils tend not to mate, so can not use mating ability...
> > > (Larson_chicken_fossil.jpg)
> > > morphological and anatomic similarities. Perhaps developmental
> > genetic species
> > > C_elegans_genetic_map.jpg (Hartwell et al., 2000, fig. 20.2)
> > > make use of genes present in a species' genome
> > > works ok for eukaryotes, tend not to work as well for prokaryotes...
> > evolutionary species (phylogenetic)
> > > Fig. 26.4
> > > But have to know about ancestors and lineage? So not an easy one to use

> prokaryotic species
> > very different, very limited morphology, number of genes varies
> > so their species definitions tend to be different from what we are considering...

Things that separate species?

One assumption of eukaryotic species is that most gene flow is vertical
from ancestors to descendants. This keeps the genomes very distinct.
Consistent with this is that different lineages are reproductively isolated,
so what are the barriers?
Fig. 24.4, reproductive barriers
(note, the text in places equates pollination with mating,
recall that pollination does not produce a zygote so it is not mating...)
note pre- and post-zygotic barriers to mating success
review the various mechanisms covered in this figure...
habitat, behavioral, and temporal isolations; mechanical and gametic isolations
reduced hybrid viability, reduced hybrid fertility, hybrid breakdown

Allopatric speciation

physical barriers between two populations of a species, in different places
allows time for divergence between those two physically separated populations,
perhaps leading to speciation if the separation lasts long enough
Fig. 24.11, (Campbell and Reece, 2002) adaptive radiation, allopatric
based on isolation and dispersion
new areas can have different natural selections, promoting divergence
land races, and variation across an environmental range often seen
these can lead to variation spread out over space, even if no distinct break?

Darwinian evolution

We can apply new terms and processes to it that he did not know about...
His model was mainly one that used allopatric speciation
It was gradualistic   no sudden shifts in character states, no "hopeful monsters"
adaptive   responds to natural selection, which leads to improved fitness
usually more fit for distinct areas, not more fit overall...
slow   not rapid, speciation occurs over millions of years
argued against catastrophism....
which would be rapid
and therefore changes would not be adaptive, due to many chance events
therefore saw environment as stable, so natural selection was stable.
most natural selection was due to competition between species and individuals...
Natural selection as his major mechanism for the divergence over time...

New additions to the model....
>Darwin could not have anticipated everything, several new features now included
>(This continual modification is based on testing, and on new observations.
>>this is consistent with Darwin's modle being a scientific model,
>>it is modifiable and testable.)

>**sympatric speciation**

A new species forms from members of a population in one location, no geographic isolation...
Several possible mechanisms for this, some involve rapid changes in ploidy or genome
>autopolyploid
>>Fig. 24.10, autopolyploidy
>>(Error in figure? Do plants make gametes by meiosis?)
>>production of new ploidy within a species
>>due to errors in meiosis by members of that species (i.e. non-disjunction, etc...)
>>this would be rapid and immediately produce a new species' ploidy number
>allopolyploid
>>Fig. 24.11, allopolyploidy
>>(Error in figure? Do plants make gametes by meiosis?)
>>contribution of two species to make a new species
>>a cross-species hybridization.
>>more likely to occur if have external fertilization?
>Endosymbiosis?
>>Fig. 25.9, diversity via endosymbiosis
>>moves entirely new genes, by horizontal gene flow... rapid change?
>There are documented examples of each of the above
>>in some cases can even directly observe these events in controlled situations

>**punctuated equilibrium**
>>Fig. 24.17, gradualism vs. punctuated model
contrast this with views of Darwin's time, and stability of environment
punctuated equilibrium suggests that sudden changes in environment alters selection
>this leads to rapid changes, due to chance... (i.e. punctuated changes)
At other times stable environment leads to constancy, this thought to account for stability in
>>fossil record and rarity of transition states (i.e. equiibrium)
>>>(from, Gould 2002)
>So stability in fossil record is real info...?
>>This is a new idea. In past fossil record seen as too limited for such evaluations.
>>>vertebrateabundance.jpg, Box 2.3, Benton (2000)
>>>mammal_evolution.jpg, Fig. 10.45, Benton (2000)
>>Can find times in the fossil record where see times of sudden speciation

This process would not be clearly adaptive or directional, based on chance?

        ex: horse evolution

            Fig. 25.25, branched evolution of horses

                a serries of chance events, leading to speciations and extinctions?

                implies that there is no clear pattern to find?...

                also not due to competition between species...

(Recall the concept of the "molecular clock" used in molecular phylogenetics? Assumed long term stability in natural selection a section of DNA would experience. This punctuated equilibrium view may through some doubt on the assumptions of the molecular clock method.)

        Rapid changes by change in developmental tempo

            (vs. Darwinian gradualism)

There are ways for rapid changes to be introduced into a species.

        a change in pattern of development, due to mutations

            Fig. 25.19, primate skull changes in development

                Darwin knew nothing about mutations....

        heterochrony: is such a change. This implies changes in controls of gene expression?

            Fig. 25.20, heterochrony, paedomorphosis

            Paedomorphosis, is retention of an earlier structure as adult, as in gills...

            alterations in homeotic genes

                changes in control of homeotic genes

                Fig. 25.21, hox gene duplication

                with new copies, the control regions in the DNA are able to change

                    and so new patterns of gene expression may become possible?

                    Ex: changes in homeotic genes in vertebrates

                        gene duplication 520 mya in Hox gene family

                        gave material for new roles in development

                  this can be a rapid change?

        With extra copies of genes, then can get new genetic combinations, produce rapid changes

            Fig. 17.6, (Gould etal, 1996) exonrecombination.jpg

            Fig. 21.14, exon shuffling

                Can take exons and reshuffle them, producing new genes rapidly

            Fig. 24.18, chromosome changes

                With polyploidy can pick and choose, and make new chromosomes

                See this in example of sunflower hybrids described in text

        These are all rapid types of speciation, faster than Darwin suggested.

            This does not preclude slow gradualistic speciation, that can happen also...

            So both rapid and slow speciation may occur.

        Other levels of selection?

Darwinism proposes selection at individual level, only

        an individual can not alter their genes, either fit enough or not...

Gene level, microevolution

  will look in next lecture at population genetics, changes in genes, or frequency of alleles at
    population level. Some argue for "selfish-genes"...
    since Darwin did not know about this level his model lacks it...

Individual level

  each individual has a set of traits, subjected to natural selection to survive to reproduce
    (Classical Darwinism covers individual and population levels)

Population level

  Traits can occur in a population that do not occur in an individual
    Chiclids are scale-eatting fish
      Fig. 23.18, scale-eating fish population
      must approach prey from the side.
        so good if not all attacks are from one side
      tend to be 50/50 right/left approaching
        grows its mouth to set the side it approaches from
        so each individual is fixed
      Fig_20_16_balanced_polymorphism_Purves.jpg (Purves et al, 1997)
      a trait of the population, not of individuals, but is adaptive?
        a balanced polymorphism in the population?
      Consider selection for sex-ratio in a population?
      See analogous situations in a population's resistance to diseases?

Higher levels of selection?

  This is still hotly debated
  Species selection species that lasts longest makes more related species
            differential speciation applied to each?
      some suggest this is why there are so many beetle species...

Summary:

So Darwinian Evolution is a model, a good start.
      Analogous to Mendelian genetics...?
      Or to description of gravity....

Now know of:

  Genetic mechanisms.
  Rapid Speciation events
  Sympatric means of speciation
  A greater role of chance events, not always adaptive or due to adaptive selection.
  Environment is not as stable as Darwin thought.
  Levels of selection other than just the individual level

But core of Modern Evolution Theory is still Darwinistic

  Allopatric speciation, mainly driven by natural selection.

But these models have been expanded, improved, so that today's version of biological evolutionary
theory is no longer purely Darwinian,

Objectives:

The term "species" is a concept that has some flexibility. How does the definition of this term depend on the circumstances, and what are the reasons for these variations? Be able to describe situations in which it would be appropriate to use different species concepts.

Darwinian evolution is a model which has been modified. What are some of the modifications that the current biological evolutionary model incorporates that Darwin's model of speciation did not take into account? Does the refinement of the model result from doubt about the fact of new species being formed, or does it reflect an acceptance of new observations concerning the mechanism(s) of speciation? Be able to describe some of the important new observations and mechanisms.

What is punctuated equilibrium, and what new claims does it make? How might gene flow occur between species? What barriers must be overcome to result in the formation of a new eukaryotic species? Be able to compare allopatric speciation and sympatric speciation. Which one can be more rapid? Which one requires more geographic space? Are all speciation mechanisms dependent on natural selection and on long periods of time?

For review, see self-quiz questions #1-6 of chapter 24.

Needed overheads and items:

Fig. 39.8, mating attempt (Purves etal. 1998)
Fig. 25.4, fossils
Larson_chicken_fossil.jpg
C_elegans_genetic_map.jpg (Hartwell etal., 2000; Fig. 20.2)
Fig. 26.4
Fig. 24.4, reproductive barriers
Fig. 24.11, (Campbell and Reece, 2002) adaptive radiation, allopatric
Fig. 24.10, autopolyploidy
Fig. 24.11, allopolyploidy
Fig. 25.9, diversity via endosymbiosis
Fig. 24.17, gradualism vs. punctuated model
vertebrateabundance.jpg, (Benton 2000; Box 2.3)
mammal_evolution.jpg, (Benton 2000; Fig. 10.45)
Fig. 24.25, branched evolution of horses
Fig. 25.19, allometric growth
Fig. 25.20, paedomorphosis
Fig. 25.21, hox gene duplication
Fig. 17.6, (Gould etal, 1996) exonrecombination.jpg
Fig. 21.14, exon shuffling
Fig. 24.18, chromosome changes
Fig. 23.18, scale-eating fish population
Fig_20_16_balanced_polymorphism_Purves.jpg (Purves et al, 1997)

Handout:

Handout - Lecture 28.stm
    Fig. 24.11, (Campbell and Reece, 2002) adaptive radiation, allopatric
    mammal_evolution.jpg, (Benton 2000; Fig. 10.45)

References:

Benton M.J- 2000- <u>Vertebrate Palaeontology</u>. 2nd edition. Blackwell Science. Oxford, U.K. Figure 10.45, and Box 2.3.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-The origin of species. Chapter 24. Pages 487-506, and pgs. 483-484, 525-531. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figure 24.11. Benjamin Cummings Press. San Francisco, CA.

Gould J.L., W.T. Keeton- 1996-<u>Biological Science</u>, 6th edition. Figure 17.6. W. W. Norton & Company. N.Y., N.Y.

Gould S.J- 2002-<u>The structure of evolutionary theory</u>. 1433 pages. Belknap Press. Cambridge, MA.

Hartwell L., L. Hood., M.L. Goldberg, A.E. Reynolds, L.M. Silver, R.C. Veres- 2000-<u>Genetics: From genes to genome</u>. Figure 20.2. McGraw Hill Press, Boston, MA.

Purves W.K., G.H. Orians, H.C. Heller, D. Sadava- 1997-<u>Life: The Science of Biology</u>. 5th edition. Figures 20.16, 39.8. Sinauer Associates Inc. Sunderland, MA.

Related issues:

Some reviews on **Darwin's model of speciation**, examples of its application, and some ways it has been altered:

> Bocxlaer I.V., S.P. Loader, K. Roelants, S.D. Biju, M. Menegan, F. Bossuyt- 2010-Gradual adaptation toward a range-expansion phenotype initiated the global radiation of toads- Science 327: (#5966, 2/5) 679-682
>
> Clutton-Brock T- 2007-Sexual selection in males and females- Science 318: (#5858, 12/21) 1882-1885
>
> Gould S.J- 2002-The structure of evolutionary theory. 1343 pages. Belknop Press. Cambridge, MA.
>
> Van Doorn G.S., P. Edelaar, F.J. Weissing- 2009-On the origin of species by natural and sexual selection- Science 326: (#5960, 12/18) 1704-1707

One rapid way to make a rapid change in a species is via **horizontal gene flow**. Here is a report of gene flow from a bacterium into several animal species. And other reports of lateral gene flow between various species of eukaryotes.

> Fukatsu T- 2010-A fungal past to insect color- Science 328: (#5978, 4/30) 574-575
>
> Hotopp J.C.D., M.E. Clark, D.C.S.G. Oliveira, J.M. Foster, P. Fischer, M.C.M. Torres, J.D. Giebel, N. Kumar, N. Ishmael, S. Wang, J. Ingram, R.V. Nene, J. Shepard, J. Tomkins, S. Richards, D.J. Spiro, E. Ghedin, B.E. Slatko, H. Tettelin, J.H. Werren- 2007-Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes- Science 317: (#5845, 9/21) 1753-1756
>
> Moran N.A., T. Jarvik- 2010-Lateral transfer of genes from fungi underlies carotenoid production in aphids- Science 328: (#5978, 4/30) 624-627
>
> Yoshida S., S. Maruyama, H. Nozaki, K. Shirasu- 2010-Horizontal gene transfer by the parasitic plant *Striga hermonthica*- Science 328: (#5982, 5/28) 1128

We now have the ability to **move entire genomes** between species.

> Pennisi E- 2010-Synthetic genome brings new life to bacterium- Science 328: (#5981, 5/21) 958-959

The **rate of speciation** need not be stable. This is the view put forward by proponents of **punctuated equilibrium**, and it has some evidence in its favor. The complexity of biotic interactions can also influence the rates of speciation.

Doebeli M., I. Ispolatov- 2010-Complexity and diversity- Science 328: (#5977, 4/23) 494-497

Krug A.Z., D. Jablonski, J.W. Valentine- 2009-Signature of the end-Cretaceous mass extinction in the modern biota- Science 323: (#5915, 2/6) 767-771

Pagel M., C. Venditti, A. Meade- 2006-Large punctuational contribution of speciation to evolutionary divergence at the molecular level- Science 314: (10/6) 119-121

Pennisi E- 2007-Variable evolution- Science 316: (5/4) 686-687

Schulte P., I. Alegret, I. Arenillas, J.A. Arz, P.J. Barton, P.R. Brown, T.J. Bralower, G.L. Christeson, P. Claeys, C.S. Cockell, G.S. Collins, A. Deutsch, T.J. Goldin, K. Gata, J.M. Grajales-Nishimura, R.A.F. Grieve, S.P.S. Gulick, K.R. Johnson, W. Kiessling, C. Koebert, D.A. Kring, K.G. MacLeod, T. Matsui, J. Melosh, A. Montanarl, J.V. Morgan, C.R. Neal, D.J. Nichols, R.D. Narris, C. Pierazzo, G. Ravizza, M. Rebolledo-Vieyra, W.U. Reimald, E. Robin, T. Saige, R.P. Speijer, A.R. Sweet, J. Urrutia-Fucugauchi, V. Vasda, M.T. Whalen, P.S. Williamsen- 2010-The Chicxulub asteroid impact and mass extinction at the Cretaceous-Paleogene boundary- Science 327: (#5970, 3/5) 1214-1218

Weir J.T., D. Schluter- 2007-The latitudinal gradient in recent speciation and extinction rates of birds and mammals- Science 315: (3/16) 1574-1576

What if a group of cells in your body decided to become a new species? They might start by becoming an **infectious disease**. This has been seen in the Tasmanian Devil, and if this disease can cross the species barrier to infect another species we would have a new **mechanism for speciation**?

Murchison E.P., C. Tovar, A. Hsu, H.S. Bender, P. Kherudpour, C.A. Rebbeck, D. Obendorf, C. Conlon, M. Bahlo, C.A. Blizzard, S. Pyecroft, A. Kreiss, M. Kellis, A. Stark, T.T. Harkins, J.A.M. Graves, G.M. Woods, G.J. Hannon, A.T. Papenfuss- 2010-The Tasmanian Devil transcriptome reveals Schwann cell origins of a clonally transmissible cancer- Science 327; (#5961, 1/1) 84-87

Here are some studies that describe the relationship between **selection** and variation at the **genomic level** which would be expected to be present before any speciation might occur.

Chamberlain N.L., R.L. Hill, D.D. Kapan, L.E. Gilbert, M.R. Kronfarst- 2009-Polymorphic butterfly reveals the missing link in ecological speciation- Science 326: (#5954, 11/6) 847-850

Wittkopp P.J., E.E. Stewart, L.L. Arnold, A.H. Neidert, B.K. Haerum, E.M. Thompson, S. Akhras, G. Smith-Winberry, L. Shetner- 2009-Intraspecific polymorphism to interspecific divergence: Genetics of pigmentation in *Drosophila*- Science 326: (#5952, 10/23) 540-544

Xia Q., Y. Guo, Z. Zhang, D. Li, Z. Xuan, Z. Li, F. Dai, Y. Li, D. Cheng, R. Li, T. Cheng, J. Jiang, C. Becquet, X. Xu, C. Liu, X. Zha, W. Fan, Y. Lin, Y. Shen, L. Jiang, T. Jensen, I. Hellmann, S. Trang, P. Zhao H. Xu, C. Yu, G. Zhang, J. Li, J. Cao, S. Liu, M. He, Y. Zho, H. Lium J. Zhao, C. Ye, Z. Du, G. Pan, A. Zhao, H. Shao, W. Zeng, P. Wu, C. Li, M. Pan, J. Li, X. Yin, D. Li, J. Wang, H. Zheng, W. Wang, X. Zhang, S. Li, H. Yang, C. Lu, R. Nielsen, Z. Zhou, J. Wang, Z. Xiang, J. Wang- 2009-Complete resequencing of 40 genomes reveals domestication events and genes in silkworm (*Bombyx*)- Science 326: (#5951, 10/16) 433-436

If you wanted to change the sexuality of a species, say to make it hermaphroditic, how hard would that be? Here is an experiment that alters just a few genes and changes nematodes into hermaphrodites. So **small genetic changes** can make huge differences leading to **speciation**.

Baldi C., S. Cho, R.E. Ellis- 2009-Mutations in two independent pathways are sufficient to create hermaphroditic nematodes- Science 326: (#5955, 11/13) 1002-1005

These articles consider **levels of selection** other than at the population level, including at the level of genes, to the level of the ecosystem. Some have considered whether cancer could be the result of selection at the cellular level.

Chuang J.S., O. Rivoire, S. Leibler- 2009-Simpson's paradox in a synthetic microbial system- Science 323: (#5911, 1/9) 272-275

Landry C.R., B. Lemos, S.A. Rifkin, W.J. Dichinson, D.L. Hartl- 2007-Genetic properties influencing the evolvability of gene expression- Science 317: (#5834, 7/6) 118-121

Li J., S. Browning, S.P. Mahal, A.M. Oelschlegel, C. Weissmann- 2010-Darwinian evolution of prions in cell culture- Science 327: (#5967, 2/12) 869-872

Mirsky S- 2009-What's good for the group- Scientific American 300; (#1, Jan) 51

Pelletier F., T. Cutton-Brock, J. Pemberton, S. Tuljapurkar, T. Coulson- 2007-The evolutionary demography of ecological change: Linking trait variation and population growth- Science 315: (3/16) 1571-1574

Shoubridge E.A., T. Wai- 2008-Side stepping mutational meltdown- Science 319: (#5865, 2/15) 914-915

Schluter D- 2009-Evidence for ecological speciation and its alternative- Science 323: (#5915, 2/6) 737-741

Smith R.J., J.T. Okano, J.S. Kahn, E.N. Bodine, S. Blower- 2010-Evolutionary dynamics of complex networks of HIV drug-resistance strains: The case of San Francisco- Science 327: (#5966, 2/5) 697-701

Yun J., C. Rago, I. Cheong, R. Pagliarini, P. Angenendt, H. Rajagopalan, K. Schmidt, J.K.V. Willson, S. Markowitz, S. Zhou, L.A. Diaz jr., V.E. Velculescu, C. Leugauer, K.W. Kinzler, B. Vogelstein, N. Papadopoulos- 2009-Glucose deprivation contributes to the development of *KRAS* pathway mutations in tumor cells- Science 325: (#5947, 9/18) 1555-1559

Zimmer C- 2006-Evolved for cancer?- Scientific American 296: (#1, Jan.) 68-75

Changes in the size of the genome often occurs during speciation, whether by **polyploidy** or other types of **mutations** in the genome. Here are some examples.

Emerson J.J., M. Cardoso-Moreira, J.O. Borevitz, M. Long- 2008-Natural selection shapes genome-wide patterns of copy-number polymorphism in *Drosophila melanogaster*-Science 320: (#5883, 6/20) 1629-1631

Gaut B.S., J. Ross-Ibarra- 2008-Selection on major components of angiosperm genomes-Science 320: (#5875, 4/25) 484-486

This review describes how the field of **medicine** is making use of evolutionary theory in the study of human disease causing agents.

Pennisi E- 2009-Darwin applies to medical school- Science 324: (#5924, 4/10) 162-163

Here are some articles on the likely roles of **heterochrony** and changes in **gene regulation** in evolution. These relate to the evolutionary-developmental connection (i.e. **evo-devo**).

Chan Y.F., M.E. Marks, F.C. Jones, G. Villameal jr., M.D. Shapiro, S.D. Brady, A.M. Southwick, D.M. Absher, J. Grimwood, J. Schmutz, R.M. myers, D. Petrov, B. Jonsson, D. Schluter, M.A. Bell, D.M. Kingsley- 2010-Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a *Pitx1* enhancer- Science 327: (#5963, 1/15) 302-305

Losos J.B- 2001-Evolution: A lizard's tale- Scientific American 284: (#3) 64-69

Losos J.B., K.I. Warheit, T.W. Schoener- 1997-Adaptive differentiation following experimental island colonization in *Anolis* lizards- Nature 387: 70-73

McNamara K.J- 2003-Importance of heterochrony- Chap. 2.3.5, pgs. 180-188, in Palaeobiology II. D.E.G. Briggs and P.R. Crowther editors. Blackwell Pub. Oxford, U.K.

Pennisi E- 2009-Ecological genomics gets down to genes - and function- Science 326: (#5960, 12/18) 1620-1621

Rebeiz M., J.E. Pool, V.A. Kassner, C.F. Aquadro, S.B. Carroll- 2009-Stepwise modification of a modular enhancer underlies adaptions in a *Drosophila* population- Science 326: (#5960, 12/18) 1663-1667

Of course, humans have evolved and are continuing to evolve, but we also influence the evolution of other species. Here is an article on **human influences on evoluton**.

Zimmer C- 2009-On the origin of tomorrow- Science 326: (#5958, 12/4) 1334-1336

Determining just what distinguishes **bacterial species**, and the major factors influencing bacterial speciation is still an area of much research, but given their short generation times it is possible to watch their actual evolution which has certain advantages.

Bohannon J- 2008-Confusing kinships- Science 320: (#5879, 5/23) 1031-1033

Fraser C., E.J. Alm, M.F. Polz, B.G. spratt, W.P. Hanage- 2009-The bacterial species challenge: Maing sense of genetic and ecological diversity- Science 323: (#5915, 2/6) 741-746

Green J.L., B.J.M. Bohannan, R.J. Witaker- 2008-Microbial biogeography: From taxonomy to traits- Science 320: (#5879, 5/23) 1039-1043

Lenski R.E., M. Travisano- 1994-Dynamics of adaptation and diversification: A 10,000-generation experiment with bacterial populations- Proceedings of the National Adademy of Science (USA) 91: 6808-6814

Retchless A.C., J.G. Lawrence- 2007-Temporal fragmentation of speciation in bacteria- Science 317: (#5841, 8/24) 1093-1096

Sheppard S.K., N.D. McCarthy, D. Falush, M.C.J. Maiden- 2008-Convergence of Campylobacter species: Implications for bacterial evolution- Science 320: (#5873, 4/11) 237-239

Of course to observe **gradual evolution** we would need to observe a species over a long time. This is possible for **bacteria**, since they have such a short life cycle allowing study of their evolution over **thousands of generations**. Here are some examples.

Barrick J.E., D.S. Yu, S.H. Yoon, H. Jeong, J.K. Oh, D. Schneider, R.E. Lenski, J.F. Kim- 2009-Genome evolution and adaptation in a long-term experiment with *Escherichia coli*- Nature 461: (10/29) 1243-1247

Harris S.R., E.J. Feil, M.T.G. Holden, M.A. Quail, E.K. Nickerson, N. Chantratita, S. Gardete, A. TaVares, N. Day, J.A. Lindsay, J.D. Edgeworth, H. DeLecastre, J. Parkhill, S.J. Peacock, S.D. Bentley- 2010-Evolution of MRSA during hospital transmission and intercontinental spread- Science 327: (#5964, 1/22) 469-474

Rainey P.B- 2009-Arrhythmia of tempo and mode- Nature 461: (10/29) 1219-1220

There are many reports of **sympatric speciation**. Here is an example.

Forbes A.A., T.H.Q. Powell, L.L. Stelinski, J.J. Smith, J.L. Feder- 2009-Sequential sympatric speciation across trophic levels- Science 323: (#5915, 2/6) 776-779

Mank J.E- 2009-Sexual selection and Darwin's mystery of mysteries- Science 326: (#5960, 12/18) 1639-1640

Some of the revisions to Darwinian speciation involve the dethroning of natural selection so that it is no longer considered to be the sole mechanism driving speciation, this happened with the **Modern Sythesis** of evolutionary theory. One of the alternative models is called the **neutral theory of speciation**.

Kimura M- 1991-Recent development of the neutral theory viewed from the Wrightian tradition of theoretical population genetics- Proceedings of the National Academy of Science (USA) 88: 5969-5973

Kimura M- 1989-The neutral theory of molecular evolution and the world view of the neutralists- Genome 31: 24-31

Kimura M- 1987-Molecular evolutionary clock and the neutral theory- Journal of Molecular Evolution 26: 24-33

Kimura M- 1981-Possibility of extensive neutral evolution under stabilizing selection with special reference to nonrandom usage of synonymous codons- Proceedings of the National Academy of Science (USA) 78: (#9) 5773-5777

Kimura M- 1979-The neutral theory of molecular evolution- Scientific American 241: (#5) 98-126

Pennisi E- 2008-Modernizing the modern synthesis- Science 321: (#5886, 7/11) 196-197

Here are some reports of cross-species **hybridization** and exchanges of genetic material, as well as how it complicates efforts to determine species relationships. The genetic basis of the hybrid incompatibility forming a barrier to speciation is now being explored.

Bayes J.J., H.S. Malik- 2009-Altered heterochromatin binding by a hybrid sterility protein in *Drosophila* sibling species- Science 326: (#5959, 12/11) 1538-1541

Brideau N.J., H.A. Flores, J. Wang, S. Maheshwari, X. Wang, D.A. Barbash- 2006-Two Dobzhansky-Muller genes interact to cause hybrid lethality in *Drosophila*- Science 314: (11/24) 1292-1295

Cattell M.V., S.A. Karl- 2004-Genetics and morphology in a *Borrichia frutescen*s and *B. arborescen*s (Asteraceae) hybrid zone- American Journal of Botany 91: (#11) 1757-1766

Gompert Z., J.A. Fordyce, M.L. Forister, A.M. Shapiro, C.C. Nice- 2006-Homoploid hybrid speciation in an extreme habitat- Science 314: (12/22) 1923-1925

Kellogg E.A., J.L. Bennetzen- 2004-The evolution of nuclear genome structure in seed plants- American Journal of Botany 91: (#10) 1709-1725

Kim M., M-L. Cui, P. Cubas, A. Gillies, K. Lee, M.A. Chapman, K.J. Abbott, E. Coon- 2008-Regulatory genes control a key morphological and ecological trait transferred between species- Science 322: (#5904, 11/14) 1116-1119

Linder C.R., L.H. Rieseberg- 2004-Reconstructing patterns of reticulate evolution in plants- American Journal of Botany 91: (#10) 1700-1708

Mihola O., Z. Trachtulec, C. Vicek, J.C. Schimenti, J. Farest- 2009-A mouse speciation gene encodes a meiotic histone H3 methyl transferase- Science 323: (#5912, 1/16) 373-375

Margulis L., D. Sagan- 2002-Acquiring Genomes: A theory of the origins of species- Basic Books, N.Y., N.Y. 240 pgs.

Mohamed S.A., F. Pirchner- 1998-Relationships between genetic distance and dominance and epistatic affects in line-crosses of mice- Journal of Animal Breeding and Genetics- 115: (#4, Aug) 313-321

Pennisi E- 2006-Two rapidly evolving genes spell trouble for hybrids- Science 314: (11/24) 1238-1239

Pfennig K.S- 2007-Facultative mate choice drives adaptive hybridization- Science 318: (#5852, 11/9) 965-967

Ritchie M.G- 2007-Feathers, females, and fathers- Science 318: (#5847, 10/5) 54-55

Saether S.A., G-P. Saetre, T. Borge, C. Wiley, N. Svedin, G. Andersson, T. Veen, J. Haavie, M.R. Servedio, S. Bureš, M. Král, M.B. Hjernquist, L. Gustafsson, J. Träff, A. Quarnström- 2007-Sex chromosome-linked species recognition and evolution of reproductive isolation in fly catchers- Science 318: (#5847, 10/5) 95-97

Tang S., D.C. Presgraves- 2009-Evolution of the *Drosophila* nuclear pore complex results in multiple hybrid incompatibilities- Science 323: (#5915, 2/6) 779-782

Willis J.H- 2009-Origin of species in overdrive- Science 323: (#5912, 1/16) 350-351

BIO 108   2010
Day 12, Lecture 29, Title: Population Genetics.
Text Readings: Campbell et al. (2008) Chapter 23.

Topics to cover:

> **Uses of population genetics**
> **Some frequency terms and their uses**
> **Hardy-Weinberg Model**
>> **Some known exceptions.**
>>> **Natural selection.**
>>> **Gene flow.**
>>> **Genetic drift.**
>>> **Mutations.**
>> **Example: effects of selection, sickle-cell anemia.**
>> **Example: multiple alleles, Human blood types.**

After considering some of the uses of population genetics, we will examine the Hardy-Weinberg model as a means to evaluate microevolutionary changes in a population. Some common exceptions to this model, and how these can extend our thinking of evolution beyond just the natural selection proposed by Darwin, will be explored.

Uses of Population Genetics
> To study evolution, population is the item that can evolve
>> gradual changes should occur in the population, can we detect it?
>> fig. 23.11, (Campbell and Reece, 2005) cline
>>> note, is this due to environment or genetics?
>>> Have to isolate genes, and compare them
>>>> How to isolate gene verision? Recall biotechnology tools?
>> Fig. 23.4, fish cline
>>> here could catch fish from different areas and compare
>>>> note determined frequency of one allele in the population
>>>> so a genetic difference exists
>> Fig. 23.3, chromosome changes
>>> here see two populations of a species of mice
>>> see differences in their chromosomes, on the path towards speciation?
> Traits found only in populations may be understood at a genetic level?
>> Fig. 23.5, caribou population distribution
>> Different populations can have distinct genetic combinations
>>> allele frequencies differ, a trait of populations
>> Balanced Polymorphisms, often involve different selection pressures...
>>> Examples: scale-eating fish, sickle-cell allele frequencies

Epidemiology   use genetic data to estimate expected number of carriers

Conservation   genetic diversity of members of a conserved species

Some frequency terms and how to determine each.

Genetic diversity is another trait of a population, has many consequences

A diploid individual can only have two alleles per gene, at most...

But a population can have many alleles per gene, dozens...

gene pool

Fig_20_1_gene_pool_Purves.jpg, fig. 20.1, Purves etal, 1998

all the types of alleles for a gene in all individuals of a population

Would be better if it were called an "allele pool"

since all members of a species may have the same genes?

How to determine allelic state?

Test cross. This takes a generation's worth of time...

examine protein variation. Need to isolate one type of protein and versions of it.

examine DNA variation. Need probes for one region of the DNA?

Frequency calculations

phenotype frequency: The proportion of a phenotype amongst the members of a population.

Red flowers and white flowers, (another example is in the text, fig. 23.7)

320 RR, 160 Rr, 20 rr (fig. 23.03a, Campbell and Reece, 2002)

f(red) = (320+160)/500 = 0.96

f(white) = 20/500 = 0.04

genotype frequency: The proportion of a genotype amongst all members of a population.

f(RR) = 320/500 = 0.64

f(Rr) = 160/500 = 0.32

f(rr) = 20/500 = 0.04

allele frequency: The proportion of a type of allele amongst all the alleles of a gene pool.

500 diploid individuals = 1000 total alleles, so have to take ploidy into account

allele frequency is not calcualted based on individuals,

but on total number of alleles

f(R) = [2(320)+160]/1000 = 800/1000 = 0.8

f(r) = [2(20)+160]/1000 = 200/1000 = 0.2

or   f(r) = 1- f(R) which is the same as $q = 1 - p$

Note assumptions of this example: just two alleles, dominant/recessive relations

life can be more complicated than this...

Hardy-Weinberg Model

Given allele frequencies in current generation, what will next generation have?

Can we detect small, microevolutionary changes?

Example of its use.

Use above allele frequencies

calculate expected genotype frequencies for next generation.

Two allele example

(fig. 23.03b, Campbell and Reece, 2002)

$f(R) = 0.8$, $f(r) = 0.2$

$f(r) + f(R) = 1.0$ which is expressed as $p + q = 1$

$f(R)^2 + 2f(r)f(R) + f(r)^2 = 1$ "   "   " $p^2 + 2pq + q^2 = 1$

$0.64 + 0.32 + 0.04 = 1$ Must add up to one...

      (fill in boxes and calculate)

Similar example in the text, see it for another view of this

      Fig. 23.7, Hardy-Weinberg example

      Note that the boxes, widths are proportional to allele frequencies

      Relate to Punnett square

          then was doing one type of cross between just one mating pair

             determined possible offspring of just that one pair

          here accounting for entire population's potential matings

          so sides of this table has frequencies of all possible gametes

             from all possible mating individuals in the population

So procedure:

      Determine current allele frequencies of a population

      Use Hardy-Weinberg equation to calculate what expected for

          next generation, this assumes no change in the population...

      Collect data for the next generation and do statistical test.

          can compare observed data to what is expected from H-W model

          typically would do comparison at level of allele frequencies

             use a Chi square test to do this...

      Microevolution: A change in allele frequency in a population.

          so above test would tell if microevolutionary change occurred

      If so: What could have caused any observed change?

      So this allows you to assess what drives microevolutionary changes.

Some known exceptions to Hardy-Weinberg Model.

Several known exceptions to the model. This model assumes no changes.

      This model is useful not because it is always correct,

          rather deviations from the model focuses our attention to look for causes

          the model also gives very percise expectations,

             making evaluation of it possible

      A nice use of a model as a null hypothesis? Refute it, as evidence of evolution.

The following are some examples of exceptions to the H-W model. That "drive"

      evolution.

Natural selection.
what if all individuals with recessive phenotype died? Strong selection
       Fig. 23.3b, (Campbell and Reece, 2002)
       320 RR and 160 Rr, all rr dead
       480 individuals now present, so now 960 alleles
       New allele frequencies now   f(R) = 0.83, f(r) = 0.17
mate selection is a type of natural selection
       Hardy-Weinberg model assumes random mating, fairly unreasonable?
       Fig. 23.15, sexual selection, peacock feathers, a clear exception
       random mating does occur more often with external fertilization
           see this in many marine animals, fungi, many plants, etc...

Gene flow.
migration of individuals between populations carry their alleles with them
       so immigration, emmigration are ways that genes flow between populations
example: if 320 RR, 160 Rr, 20 rr in a population, and then another 20 rr arrive
       what will be the new allele frequencies?
           f(R) = 0.77, f(r) = 0.23
           tell students to confirm this on their own...

Genetic drift.
       fig. 23.08, genetic drift
due to small population sizes, compare to coin tossing
       note effect of chance on offspring in next generation
can be produced by anything that forms a small isolated population
       fig. 23.9, bottleneck effect
       example: bottleneck effect
           due to massive hunting, glaciers, etc.
           only a small number of survivors, their alleles are passed on
       Fig. 50.1, (Campbell and Reece, 2005) seal population
           Fur seals, survivors of a bottle neck effect
           consequence is a very low genetic diversity
               homozygous for most of its genes
               makes population less genetically variable
                 and so more vulnerable
       Another example is the founder's effect,
           a new population formed by a small initial number of individuals

Mutations.

for eukaryotes, this is the ultimate source of variation, but we say it is rare

    obviously sexual recombination works with variations made by mutation

        1 mutation/10^8/gene/individual generation

        given about 30000 genes per humans in germline cells

        0.0003 chance of mutation per individual generation

            even so, may be a silent mutation...

        each generation takes years... so do not expect to see many soon

    but with enough time or individuals, or somatic cells will see variation

for prokaryotes, also ultimate source of variation

        1 mutation/10^6/gene/individual generation

        about 3000 genes per bacterium

        0.003 change of mutation per individual generation

        (0.003)(48) generations per day = 0.144 mutations per day

            this works because their generation times are so short

        If start with culture with millions of bacteria will get mutations.

So if assume a short enough time, or a few enough generations,

    then mutations are not likely to greatly alter the allele frequencies

Note that high rates of mutation can be a strategy

    Gago etal - mutation rates - 2009.jpg

    In viruses the mutation rates are much higher!

    This produces very diverse viral populations

        allowing them to evade our immune system

We use this H-W model as a tool to estimate what will happen if there are no evolutionary changes. Then we examine actual data, and if detect differences from what the model predicts we can explore how the changes are made to determine aspects of items driving that microevolution.

So the Hardy-Weinberg Model:

    $p + q = 1$

    $p^2 + 2pq + q^2 = 1$ (a binomial expansion)

    Assumes

        just two alleles per gene (classical Mendelian types)

        no natural selection (i.e. random mating)

        no gene flow (i.e. no immigaration/emmigration)

        no genetic drift (i.e. large population size)

        no net mutation pressure (i.e. not a huge number of generations)

    Other exceptions to this model exist, the above are the major assumptions...

An example of the effects of selection: sickle-cell anemia.
Here will give a simple example that uses the Hardy-Weinberg model
       to examine human microevolution.
       Fig. 23.17, malaria distribution
              Note the frequency of sickle-cell allele (s) does vary, from 0->12.5%
       Fig. 5.22, sickle red blood cells and change in amino acid for it
              the hydrophobic hemoglobins form long chains and distort the cell shapes
       Fig. 14.15, (Campbell and Reece, 2002) sickle-cell effects
       recall sickle red blood cells and effects on phenotype, and relate to genotypes
              SS - is wildtype, normal red blood cells, vulnerable to malaria
              Ss - is heterozygous, some sickle red blood cells some normal, resistant to malaria
                    some call this "sickle cell trait"
              ss - all red blood cells are sickle cells, resistant to malaria, has sickle cell disease
       Example of human populations in the presence of malaria

		Given initial allele frequency	Observed in presence of malaria		
Genotype	allele freq.	expected genotype frequency	observed genotype frequency	Selection on phenotypes	new allele freq.
SS	f(S) = 0.9	8100	7962	0.98	f(S) = 0.8973
Ss	f(s) = 0.1	1800	2022	1.12	
ss		100	16	0.16	f(s) = 0.1027
	total population	10000	10000		

In the above hypothetical population assumed an initial f(S) = 0.9, f(s) = 0.1
       Note the use of H-W model to calculate what would be expected in next generation.
       Note the comparison to the observed genotypes.
       Can determine the selection applied to phenotypes?
              note heterozygote advantage of Ss genotype in presence of malaria
              Selection for malarial resistance, selection against allele
                    this can lead to a balance of conflicting selection pressures
                    producing a balanced polymorphism...
       Suggests a microevolutionary change,
              could use Chi square test to see if it was significant...
So in areas with malaria the sickle-cell allele persists, while in absence of malaria it is strongly selected against.
       An example of a balanced polymorphism will occur in areas of malaria...

An example using multiple alleles: Human blood types.

        Have only considered model with two allele per gene, what if more?

        Three allele example seen in human Blood Types

                Fig. 14.11, human blood types

                    alleles: I^A, I^B, i

                In this case one gene has three allelic versions, only two in any one person.

        So in this case Hardy-Weinberg model is:

                For allele frequencies: $f(I^A) + f(I^B) + f(i) = 1$

                                                  $p + q + w = 1$

                For genotype frequencies: $I^{A2} + 2I^AI^B + 2I^Ai + 2I^Bi + I^{B2} + i^2 = 1$

                                                  $p^2 + q^2 + w^2 + 2pq + 2pw + 2qw = 1$

                Show this as a Punnett square with three columns and three rows...

                        instead of a binomial expansion, it is a trinomial expansion...

                To calculate phenotype frequencies for each blood type:

                      f(Type A) = $p^2 + 2pw$

                      f(Type B) = $q^2 + 2qw$

                      f(Type AB) = $2pq$

                      f(Type O) = w^2

            What if: $f(I^A) = 0.4$, $f(I^B) = 0.4$, $f(i) = 0.2$

                      Could students calculate phenotype frequencies?

Summary

        Use of allele frequencies in population as one measure of genetic structure of a population

        Changes in allele frequencies over time is evidence of microevolution.

        Can use this via Hardy-Weinberg model to calculate:

                Selection

                frequency of carriers of a genetic condition

                population size needed to conserve a species' genetic diversity

        This shows the usefulness of the violation of a model to help us focus our thinking...

                A use of the null hypothesis in a good way...

Objectives:

        Given a set of genetic data of a population you should be able to determine the allele, genotype, or phenotype frequencies, and if given sets of data taken at different times you should be able to determine whether or not the population is in Hardy-Weinberg equilibrium. What are some typical exceptions to the Hardy-Weinberg model, and which of these exceptions are not accounted for by the model of evolution as it was originally proposed by Darwin? Which of these exceptions are often adaptive, and which produce random changes that may or may not be adaptive?

        In terms of a population's genetics what must happen in order for that population to evolve? According to the Hardy-Weinberg model what are several ways in which a population can carry out this evolution, which ways are more rapid and which will require the most time, which could make the largest changes in the least amount of time?

For review, see self-quiz questions #1-5 of chapter 23.

Needed overheads and items:

Fig. 23.11, (Campbell and Reece, 2005) cline
Fig. 23.4, fish cline
Fig. 23.3, chromosome changes
Fig. 23.5, caribou population distribution
Fig_20_1_gene_pool_Purves.jpg, fig. 20.1, (Purves etal, 1998)
Fig. 23.3a, (Campbell and Reece, 2002)
Fig. 23.3b, (Campbell and Reece, 2002)
Fig. 23.7, Hardy-Weinberg example
Fig. 23.3b, (Campbell and Reece, 2002)
Fig. 23.15, sexual selection
Fig. 23.8, genetic drift
Fig. 23.9, bottleneck effect
Fig. 50.1, (Campbell and Reece, 2005) seals
Gago etal - mutation rates - 2009.jpg
Fig. 23.17, sickle cell allele frequencies
Fig. 5.22, red blood cells and sickle cell
Fig. 14.15 (Campbell and Reece, 2002) sickle cell anemia
Fig. 14.11, human blood types

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-The evolution of populations. Chapter 23. Pages 468-486. Biology. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-Biology, 7th edition. Fig. 50.1. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-Biology, 6th edition. Figures 14.15, 23.3. Benjamin Cummings Press. San Francisco, CA.

Gago S., S.F. Elena, R. Flores, R. Sanjuán- 2009-Extremely high mutation rate of a hammerhead viroid- Science 323: (#5919, 3/6) 1308

Purves W.K., G. H. Orians, H.C. Heller, D. Sadava- 1998-Life: The Science of Biology, 5th Edition. Figure 20.1. Sinauer Associates Inc. Sunderland, MA.

Related issues:

The Hardy-Weinberg model can be violated by **natural selection**. But if the selection is stabilizing, then this may lead to **balanced polymorphisms** which would show no net change over time. Sexual selection can also occur, and to be able to induce evolutionary changes.

Chaine A.S., B.E. Lyon- 2008-Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting- Science 319: (#5862, 1/25) 459-462

Seidel H.S., M.V. Rockman, L. Kruglyak- 2008-Widespread genetic incompatibility in *C. elegans* maintained by balancing selection- Science 319: (#5863, 2/1) 589-594

Severinghaus L.L., H-C. Lin- 1990-The reproductive behaviour and mate choice of the fiddler crab (*Uca lactea lactea*) in Mid-Taiwan- Behaviour 113: (#3-4) 292-307

This article considers the effects of **genetic drift** and **small population sizes** on microevolution, as it describes the reconsideration of one example long thought to be due to genetic drift.

Pennisi E- 2007-Natural selection, not chance, paints the desert landscape- Science 318: (#5849, 10/19) 376

Here are studies of the **Founder's effect**. This concept has been applied to viral populations in an individual. The other article considers small fruit fly populations in Hawaii.

Bhattacharya T., M. Daniels, D. Heckerman, B. Foley, N. Frahm, C. Kadie, J. Carlson, K. Yusim, B. McMahon, B. Gaschen, S. Mallal, J.I. Mullins, D.C. Nickle, J. Herbeck, C. Rousseau, G.H. Learn, T. Msura, C. Brander, B. Walker, B. Korber- 2007-Founder effects in the assessment of HIV polymorphisms and HLA allele associations- Science 315: (3/16) 1583-1586

DeSalle R., A.R. Templeton- 1988-Founder effects and the rate of mitochondrial DNA evolution in Hawaiian *Drosophila*- Evolution 42: (#5) 1076-1084

The influence of **mutation** rates and new mutations on microevolution is of interest. One article below notes how mutation has led to changes in pigmentation. Another deals with the mutation that led to lactose intolerance recently in humans, which relates to who drinks milk as adults. Another is a good review of new mutations found in humans.

Drayna D- 2005-Founder mutations- Scientific American 293: (#4, Oct.) 78-85

Gibbons A- 2006-There's more than one way to have your milk and drink it too- Science 314: (12/15) 1672

Hoekstra H.E- 2006-Genetics, development and evolution of adaptive pigmentation in vertebrates- Heredity 97: 222-234

Here are some examples of studies of various phenotypes and attempts to determine the genetic factors in the population involved in their production. Often this involves uses of the **Hardy-Weinberg model**, or models modified from it, to follow the **microevolutionary changes** in populations.

Cook L.M., B.S. Grant- 2000-Frequency of *insularia* during the decline in melanics in the peppered moth *Biston betularia* in Britain- Heredity 85: 580-585

Gratten J., A.J. Wilson, A.F. McRae, D. Beraldi, P.M.Visscher, J.M. Pemberton, J. Slate- 2008-A localized negative genetic correlation constrains microevolution of coat color in wild sheep- Science 319; (#5861, 1/18) 318-320

Here is a nice text that shows some of the ways that the **Hardy-Weinberg model** has been expanded and used in a wide variety of applications.

Hedrick P.W- 2000-<u>Genetics of Populations</u>- 2nd edition, 500 pgs. Jones and Bartlett Publishers. Sudbury, MA.

With the sequencing of the **human genome** there is now discovery of areas in it that are under positive selection by analysis of variation in the DNA sequences. Some studies claim to find regions of our genome that are under **positive selection**. For example the selection in humans for **malarial resistance**. Also below is a study of how the use of human genetic diversity allows us to estimate past patterns of **human migration** through asia.

Barreiro L.B., G. Laval, H. Quach, E. Patin, L. Quintana-Murci- 2008-Natural selection has driven population differentiation in modern humans- Nature Genetics 40: (#3, March) 340-345

Hugo Pan-Asian SNP Consortium- 2009-Mapping human genetic diversity in Asia- Science 326: (#5959, 12/11) 1541-1545

Louicharoen C., E. Patin, R. Paul, I. Nuchprotyoon, B. Witoonpanich, C. Peerapittayamongkol, I. Casademont, T. Sura, N.M. Laird, P. Singhasivanon, L. Quintana-Murci, A. Sakuntabhai- 2009-Positively selected G6PD-mahidol mutation reduces *Plasmodium vivax* density in South East Asians- Science 326: (#5959, 12/11) 1546-1549

Sabeti P.C., P. Varilly, B. Fry, J. Lohmueller, E. Hostetter, C. Cotsapas, X. Xie, E.H. Byrne, S.A. McCarroll, R. Gaudet, S.E. Schaffner, E.S. Lander, The International Map Consortium- 2007-Genome-wide detection and characterization of positive selection in human populations- Nature 449: (Oct. 18) 913-918

BIO 108   2010
Day 12, Lecture 30, Title: Pollination Ecology.
Text Readings: Campbell et al., (2008), pgs. 630-632, 804-805, 1198-1203.

Topics to cover:

What is Pollination?
Pollination Strategies
    Species-species interactions

What is Pollination?
    Many people mistake pollination for sex or for dispersal.
    Pollination is NOT fertilization.
        No gametes are fused during pollination. No zygote is formed.
        Just the movement of pollen grains (male gametophytes) from
            one location to another.
    Pollination is also NOT dispersal.
        Dispersal is movement to a new location, where species previously not found.
        Pollination has to get pollen grains to other members of same species.

Pollination Strategies
    Want to keep track of types of pollination strategies.
        Does the plant make use of animal pollinators or not?
        What types of pollinators are there? Which animal groups involved?
        What types of modifications do plants have to influence pollinators?
    What types of interactions are occurring between plants and animals?
        What are the costs to plants of the various strategies?
            Cost of rewards to the pollinator.
        What benefits are there to animals that act as pollinators?
            In addition to food, what other benefits are often obtained?

    Species-species interactions
    Pollination is often done via species-species interaction,
        which is an aspect of ecology.
        Typically a type of mutualism.
            both the plant and the animal involved benefit
            but are costs as well
                cost of nectar?
        Some interactions involve plant being parasitic on animals
            sexual deception gives no benefit to the animal?

Objectives:

Describe several strategies that flowering plants use to achieve pollination. What modifications to what parts of the plant are associated with each of these strategies? What costs to the plant does each strategy impose? Be able to describe several of the benefits to those animals that act as pollinators. What do some of these animals gain? Gain an appreciation of examples of plant/animal interactions. Be able to classify pollinator/plant interactions in terms of those given in the table on pg 1219 (in the review section of chapter 54).

Is the pollination of flowering plants fully dependent on animals or is it every achieved by abiotic means? How would the flowers of such plants differ from those that use pollinators? What are several examples of plants that use abiotic pollination?

Be able to describe the differences between pollination and fertilization, and the differences between pollination and dispersal as seen in the flowering plants.

For review, see self-quiz question #1 of chapter 38.

Needed overheads and items:

Video player
     Video tape   (popcorn optional....)

References:

Attenborough D- 1995-The Private Life of Plants. Vol. 3: *The Birds & The Bees*.
British Broadcasting Corporation.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky,
R.B.Jackson- 2008-Biology. Eighth edition. Pgs. 630-632, 804-805, 1198-1203.
Pearson/Benjamin Cummings Press. San Francisco, CA.

Related issues:

Flowering plants use diverse pollination strategies. Here are some reports looking at **biotic pollination** means.

Fetscher A.E., J.R. Kohn- 1999-Stigma behavior in *Mimulus aurautiacus* (Scrophulariaceae)- American Journal of Botany 86: (#8) 1130-1135

Hodges S.A- 1997-Floral nectar spurs and diversification- International Journal of Plant Science 158: (#6S) S81-S88

Johnson S.D., A. Ellis, S. Dötterl- 2007-Specialization for pollination by beetles and wasps: The role of lollipop hairs and fragrance in *Satyrium microrhynchum* (Orchidaceae)- American Journal of Botany 94: (#1) 47-55

Johnson S.D., A. Pauw, J. Midgley- 2001-Rodent pollination in the African lily *Massonia depressa* (Hyacinthaceae)- American Journal of Botany 88: (#10) 1768-1773

Johnson S.D., J.J. Midgley- 1997-Fly pollination of *Gorteria diffusa* (Asteraceae), and a possible mimetic function for dark spots on the capitulum- American Journal of Botany 84:   (#4) 429-436

Kawakita A., M. Kato- 2004-Evolution of obligate pollination mutualism in New Caledonian *Phyllanthus* (Euphorbiaceae)- American Journal of Botany 91: (#3) 410-415

Kessler D., K. Gase, I.T. Baldwin- 2008-Field experiments with transformed plants reveal the sense of floral scents- Science 321: (#5893, 8/29) 1200-1202

Koopowitz H., T.A. Marchant- 1998-Post pollination nectar reabsorption in the african epiphyte *Aerangis verdickii* (Orchidaceae)- American Journal of Botany 85: (#4) 508-512

Martins D.J., S.D. Johnson- 2007-Hawk moth pollination of aerangoid orchids in Kenya, with special reference to nectar sugar concentration gradients in the floral spurs- American Journal of Botany 94: (#4) 650-659

Muchhala N- 2006-The pollination of *Burmeistera* (Campanulaceae): Specialization and syndromes- American Journal of Botany 93: (8) 1081-1089

Neiland M.R.M., C.C. Wilcock- 1998-Fruit set, nectar reward, and rarity in the Orchidaceae- American Journal of Botany 85: (#12) 1657-1671

Sakai S., M. Kato, H. Nagamasu- 2000-*Artocarpus* (Moraceae) gall midge pollination mutualism mediated by a male-flower parasitic fungus- American Journal of Botany 87: (#3) 440-445

Sakai S., T. Inoue- 1999-A new pollination system: Dung-beetle pollination discovered in *Orchidentha inouei* (Lowiaceae, Zingiberales) in Sarawak, Malaysia- American Journal of Botany 86: (#1) 56-61

Steiner K.G., V.B. Whitehead, S.D. Johnson- 1994-Floral and pollinator divergence in two sexually deceptive South African orchids- American Journal of Botany 81: (#2) 185-194

Ushimaru A., T. Watanabe, K. Nakata- 2007-Colored floral organs influence pollinator behavior and pollen transfer in *Commelina communis* (Commelinaceae)- American Journal of Botany 94: (#2) 249-258

Pollen can also complete with each other. Here is an article about **pollen competition**.

Armbruster W.S., D.G. Rogers- 2004-Does pollen competition reduce the cost of
inbreeding?- American Journal of Botany 91: (#11) 1939-1943

In general we see **gymnosperms** as using abiotic pollination strategies, which seems to suggest that they do nothing to ensure pollination. But that is not always true. Here is one study that reports the biotic pollination strategy of a cycad, and two others that describe thes use of a sticky "pollination droplet" to catch pollen.

Takaso T., J.N. Owens- 1996-Ovulate cone, pollination drop, and pollen capture in
Sequoiadendron (Taxoidiaceae)- American Journal of Botany 83: (#9) 1175-
1180

Terry I., G.H. Walter, C. Moore, R. Roemer, C. Hull- 2007-Odor-mediated push-pull
pollination in cycads- Science 318: (#5847, 10/5) 70

Tomlinson P.B., J.E. Braggins, J.A. Rattenbury- 1997-Contrasted pollen capture
mechanisms in Phyllocladaceae and certain Podocarpaceae (Coniferales)-
American Journal of Botany 84: (#2) 214-223

These articles suggests the use of pollinators by plants well before there were angiosperms.

Ollerton J., E. Coulthard- 2009-Evolution of animal pollination- Science 326: (#5954, 11/6)
808-809

Ren D., C.C. Labandeira, J.A. Santiago-Blay, A. Rasnitsyn, C. Shih, A. Bashkuev, A.V.
Login, C.L. Hotton, D. Dilcher- 2009-A probable pollination mode before
angiosperms: Eurasian, long-proboscid scorpionflies- Science 326: (#5954, 11/6)
840-847

Insects do not just help plants with pollination, there are other plant/insect interactions. In some cases there are reports that insects help with the transfer of sperm as well, as in this case about animals helping with the transfer of **moss sperm**.

Cronberg N., R. Natcheva, K. Hedlund- 2006-Microarthropods mediate sperm transfer in
mosses- Science 313: (9/1) 1255

Some flowering plants have a mechanism to pervent the pollen released from one plant from growing down the style of the same plant. This is called **self-incompatibility**, and is a means to promote the success of cross-pollinated pollen. Others use a strategy of promoting the success of pollen released from the same plant.

Dixit R., J.B. Nasrallah- 2001-Recognizing self in the self-incompatibility response- Plant Physiology 125: 105-108

Lipow S.R., S.B. Broyles, R. Wyatt- 1999-Population differences in self-fertility in the "self-incompatible" milkweed *Asclepias exaltata* (Asclepiadaceae)- American Journal of Botany 86: (#8) 1114-1120

Tang C., C. Toomajin, S. Sherman-Broyles, V. Plagnol. Y-L. Guo, T.T. Huo, R.M. Clark, J.B. Nasrallah, D. Weigel, M. Nordberg- 2007-The evolution of selfing in *Arabidopsis thaliana*- Science 317: (#5841, 8/24) 1070-1072

Pollination can do more than just get the pollen to the stigma. Many other **signals** can be brought that can be received by the carpel and the ovules.

O'Neill S- 1997-Pollination regulation of flower development- Annual Review of Plant Physiology and Plant Molecular Biology 48: 547-574

BIO 108   2010
Day 13, Lecture 31, Title: Origin(s) of Life.
Text Readings: Campbell et al. (2008), pgs. 58-59, 507-525.

Topics to cover:

>   **Contrasting the origin of life and evolution of life.**
>   **What must a biological model of the origin of life do?**
>   **What is needed for a model of the origin of life?**
>   **How to get carbon chemistry and organic matter**
>   **Compartmentation**
>   **catalysis/metabolism coupled to energy flow**
>   **information content/flow/control**
>   **So the model**...

Contrasting the origin of life and evolution of life.
First: Biological evolutionary theory assumes life already exists
>   so the model of biological evolution is NOT an attempt to explain the origin of life.
>   Biological evolution is an emergent property OF life; so need life to do it.
>   Models of the origins of life are distinct from models of biological evolution.
Old views
>   vital essence. Item least well known thought to hold a vital "spark"....
>   spontaneous biogenesis
>   Fig. 26.9, (Campbell and Reece, 2002) Pasteur and spontaneous generation
>   was a well accepted idea in the 19th century
>   tested by Pasteur
>   Fig. 26.9, (Campbell and Reece, 2002) PaseturMicroorg
>   So under these conditions spontaneous generation must be rare
>   this refutes vitalism?
>   Consistent with a common heritage for life so Darwinists were happy
>   But this puts origin of life, under these conditions, in a tough spot?

What must a biological model of the origin of life do?
>   A model does not provide proof... And can not answer all questions...
>   A good model must give a plausible, and testable, framework
>   analogy with the game of chess? (From Feynman)
>   So need steps proposed, and each step must be testable.
>   This does not mean that events had to have happened this way,
>   a model is limited...
>   The model must account for an origin of a prokaryotic organism.
>   From that first common ancestor can then use biological evolution mechanisms
>   to get all the other known species of life.

What is needed for a model of the origin of life?
A model would need to account for at least four features of earliest life.
> Four classes of organic matter should be present. Can these be made?
> Compartmentation. Cells have a distinct inside and outside. So need membranes.
> Catalysis/metabolism coupled to energy flow. Need to have enzymatic activities.
> Information content/flow/control. Something has to act to carry heritable information.

1) **How to get carbon chemistry and organic matter**
> Fig. 1 (pg. 18), (Zubay, 2000) solarelements.jpg
> So C, N, O are available in the solar system. This was not true in first solar systems?
>> Can make stable bonds, so can make stable organic matter (OM) from them.
> Could organic matter be made on the early Earth?
> Urey-Miller experiment.
>> Fig. 4.2, Urey-Miller experiments
>>> Makes most OM if done with a reducing atmosphere.
>>> Can get amino acids, simple sugars, N-bearing bases....
>>> Consider what this did NOT demonstrate?...
>> Fig. 26.10x, (Campbell and Reece, 2002) lightning
>>> do get significant amounts of chemical changes from lightning
>>> Other energy sources tried include UV light, heat, radioisotopic radiation...
>> Pathways to glycine production, example of options
>>> if several paths to the same end, then likely to be made...?
>> Table 1 (pg. 174), (Zubay, 2000) glycineformation.jpg
>>> note that initial conditions matter!
>>> oxygen presence is best, but not likely to be present early on.
>>> can be done in presence of water, and under neutral to reduced conditions
> Must all the OM have been made on Earth?
>> OM in space has been detected
>>> horseheadnebula.jpg (Sagan, 1994; pg. 21)
>> OM in nebulae and in meteorites that have come to earth.
>> So is formed in space as well?
> So either make organic matter here on earth, and/or get it from space...
> How then to get polymers? Use condensation reactions.
>> Fig. 5.2, condensation reaction
>> Many ways to drive such reactions by pulling out the water...
>>> think tidal pools, so a role for the moon and tides?
>>> think water removal by freezing
>> Obviously this implies a planet with a strong moon, or strong seasonal changes
>>> in the proper temperature ranges.
> Keeping OM stable on earth.
>> Ultraviolet light from the sun disrupts bonds in OM.
>> So OM would accumulate in water which absorbs UV well?

So far the good news:

No type of OM has been found that can NOT be made abiotically.

so no "vital essence" in these molecules...

Often have several ways to make the same type of OM...

Polymerization can occur fairly easily

So on an early earth, lacking oxygen gas,

these organic molecules would accumulate

2) **Compartmentation**

A means to concentrate and retain items is essential.

Life has self, versus non-self... Will consider some possible compartments.

Clays as a possible first form?

clay.jpg (Raven and Johnson, 1993; fig. 4.10)

charges on clay interact with OM in specific ways. Can concentrate some OM.

Once lipids are formed, vesicles form spontaneously, with membranes.

Coacervate1.jpg (Raven and Johnson, 1993; fig. 19.2)

This can separate inner vs. outer hydrophilic compartments.

OM extracted from meteorites include hydrophobic ones

when these placed in water they form coacervate droplets, vesicles

coacervate2.jpg (Keeton and Gould, 1993; fig. 19.2)

Hydrophobic Amino acids, polymerized by dehydration cycles

Fig. 19.6, (Gould etal, 1996) proteinoidmicrospheres.jpg

will form hydrophobic proteins

these proteins can create proteoid microspheres spontaneously

Now a hydrophobic surface barrier arround a hydrophilic interior, see other properties

concept of reproduction of lipid vesicles

decoupled from replication of molecules

Then need to be able to move things across the barrier...

Some of the hydrophobic proteins made this way have ion channel activity!

3) **Catalysis/metabolism coupled to energy flow**

Catalysis can be done by many types of OM

proteins, DNA, RNA, carbohydrates, lipids

Ribozymes

ribozyme.jpg (Purves et al., 1998, fig. 24.5)

RNAs with catalytic ability, act in many central reactions in life

involved in life in peptide bond formation

modification of bonds in nucleic acids... in splicing, etc...

ribozymetypes.jpg (Zubay, 2000; table 3)

So could see ribozymes early in life.

Want to couple desired reactions to $-\Delta G$ reactions

Some of these include oxidation reactions such as:

$$NH_3 \rightarrow NO_2, \quad CH_4 \rightarrow CO_2$$
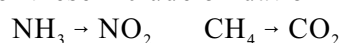
Energy also available in other forms, but may be too much?
        ever tried to control a lightning bolt?
        UV radiation?
        these are perhaps OK to make OM, but tough as a first energy source?
Mineral cycles could be coupled to?
        Sulfur redox cycle, also iron redox cycle, examples found today
        Fig. 24.7, (Purves etal, 1998) sulfurredox.jpg
Lots of evidence of no free oxygen gas in early earth
        Of course, lots of aerobic life couple their reactions to oxidation of OM
            But free oxygen gas not available early on, early on was anaerobic
        types of minerals formed under anaerobic conditions differs from under aerobic
            therefore can detect redox state from rock strata, see rusting of earth
        oxygen gas does not promote OM stability, from thermodynamic point of view
            we know it will all fall apart unless balanced by rates of synthesis...
        Types of gases coming out of earth are often reduced
        Hydrothermal vents as a source of such reduced items
        (Cone, 1991) hydrothermalvents.jpg
        fig. 25.2, hydrothermal vent
        Arguments exist over whether H_2 might have been present in early atm.
            (Tian et al, 2005)
Would then couple positive free energy changes to negative free energy changes seen
        from redox reactions. Chemolithotrophy.
Can trap metabolism in vesicles
        Fig. 25.3, varients of protobionts, liposomes
        Vesicle can reproduce
        but if the vesicle blebs the catalysts will be diluted?

4) **Information content/flow/control**
        So reproduction of whole structure is not sufficient
            need replication of essential molecules in structure
            this is an essential metabolic feature. So need catalysis in a vesicle-like structure?
        RNA shows ability to do catalysis and guide replication
        Ribozymes
            Splicesomes have ribozymes
            Ribosomes have ribozymes
                peptide bond formation is catalyzed by a ribozyme
                this must be the most important reaction for life
                many types still found in life tocay
        Can do *in vitro* selection for RNA
            Fig. 26.11, (Campbell and Reece, 2002) abiotic RNA replication?
            for self replication, and for catalytic ability
        Suggestions made of early "RNA world"
            RNA acts as a catalyst and as holder of genetic information.

Early RNA world, later proteins took over catalysis, DNA for information storage
        RNA kept in between?
        Fig. 26.13, (Campbell and Reece, 2002) RNA and membrane compartmentation
        so perhaps ribozymes caught in vesicles?
        if can code for their own replication then will not be diluted out
        Better than proteins, since can self-replicate?
        Better than DNA, as more often do catalysis?

How might these nucleotides have gotten organized in the first place?
Some propose that the information content in minerals
        might have helped guide early OM (Cairns-Smith, 1982)
        minerals have handedness, could select for amino acid handedness
                can help promote certain OM reactions

So the model
        OM abundant from abiotic means
                either on earth or from space
        polymerization can occur abiotically due to tides
        Energy sources exist, some gentle enough for early life to use
        Compartments can form spontaneously so long as we
                have water, polymerization, and hydrophobic molecules
        Catalysis abilities found broadly.
        Information control may have first occurred in RNA
                later replaced by DNA as a backup
                and catalysis mostly taken over by proteins

        This is NOT proof. But it is the sketch of a model
                for the origin of life. Work continues.
        Can use this information to anticipate the types of planets on which life might
                be present?

Objectives:

What energy sources were available to promote the formation of complex organic molecules from inorganic constituents? How might polymerization have been achieved?

Miller and Urey did several experiments using what they estimated to be conditions found on the early earth. What did they do and what did they conclude? What evidence supports their estimates of the early conditions on earth?

What are hydrothermal vents and what advantages do they offer as a site for the early origins of life? Having catalysts is essential, but the catalysts must be capable of coupling reactions. Why is this so, and what types of reactions should they be able to couple? What are ribozymes? What properties do they have that make them an interesting candidate as an early genetic element?

How easily do vesicles form? What are advantages and disadvantages of having membranes present during the formation of life?

Replication and reproduction are seen in life, and are also seen in some non-living systems. How do these two processes differ? Must replication and reproduction always be tightly coupled, or can they occur separately? What are examples of abiotic systems that show replication, or reproduction, and yet are not alive?

Abiotic chemistry is essential to form many of the molecules needed for the origin of life. Should a biologist argue that these abiotic processes must be accounted for by the biological theory of evolution? How do biologists account for the failure to find life originating spontaneously today? How might this argument also account for the observation of just one clade of life on earth today rather than several distinct clades of life?

Needed overheads and items:

Fig. 26.9, (Campbell and Reece, 2002) Pasteur and spontaneous generation
Fig. 26.9, (Campbell and Reece, 2002) PaseturMicroorg
Fig. 1 (pg. 18), (Zubay, 2000) solarelements.jpg
Fig. 4.2, Urey-Miller experiments
Fig. 26.10x, (Campbell and Reece, 2002) lightning
Table 1 (pg. 174), (Zubay, 2000) glycineformation.jpg
horseheadnebula.jpg (Sagan, 1994; pg. 21)
Fig. 5.2, condensation reaction
clay.jpg (Raven and Johnson, 1993; fig. 4.10)
Coacervate1.jpg (Raven and Johnson, 1993; fig. 19.2)
coacervate2.jpg (Keeton and Gould, 1993; fig. 19.2)
Fig. 19.6, (Gould etal, 1996) proteinoidmicrospheres.jpg
ribozyme.jpg (Purves et al., 1998, fig. 24.5)
ribozymetypes.jpg (Zubay, 2000; table 3)
Fig. 24.7, (Purves etal, 1998) sulfurredox.jpg
(Cone, 1991) hydrothermalvents.jpg
Fig. 25.2, hydrothermal vent
Fig. 25.3, varients of protobionts, liposomes
Fig. 26.11, (Campbell and Reece, 2002) abiotic RNA replication?
Fig. 26.13, (Campbell and Reece, 2002) RNA and membrane compartmentation

Handout:
Handout - Lecture 31.stm
    Table 1 (pg. 174), (Zubay, 2000) glycineformation.jpg
    ribozymetypes.jpg (Zubay, 2000; table 3)
    Fig. 26.11, (Campbell and Reece, 2002) abiotic RNA replication?
    Fig. 26.13, (Campbell and Reece, 2002) RNA and membrane compartmentation

References:

Cairns-Smith A.G- 1982-<u>Genetic takeover and the mineral origins of life</u>. Cambridge
  University Press, Cambridge.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky,
  R.B.Jackson- 2008-The history of life on Earth. Chapter 25. Pages 507-525, and
  58-59. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San
  Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figures 26.9, 26.10, 26.11, 26.13.
  Benjamin Cummings Press. San Francisco, CA.

Cone J- 1991-<u>Fire under the Sea</u>- William Morrow and Company, Inc. N.Y., N.Y.

Keeton W.T., J.L. Gould-1993-<u>Biological Science</u>, 5th edition. Figures 19.2, 19.6. W.W.
  Norton & Company. N.Y., N.Y.

Purves W.K., G.H. Orians, H. C. Heller, D. Sadava- 1998-<u>Life: The Science of Biology</u>.
  5th edition. Figures 24.5, 24.7. Sinauer Associates, Inc. Sunderland, MA.

Raven P.H., G.B. Johnson- 1992-<u>Biology</u>, 3rd edition. Figures 4A, 4.10. Wm.C. Brown
  Publishers. Dubuque, Iowa.

Sagan C- 1994-<u>Pale Blue Dot</u>- pg. 21. Random House, N.Y., N.Y.

Tian F., O.B. Toon, A.A. Pavlov, H. DeSterck- 2005-A hydrogen-rich early earth
  atmosphere- Science 308: (5/13) 1014-1017

Zubay G- 2000-<u>Origins of Life on the Earth and in the Cosmos</u>. 2nd edition. Tables
  1 and 3, Figure 1. Academic Press. San Diego, CA.

Related issues:

Here are some nice **texts** that cover various issues relating to the **origin of life**. I especially recommend the ones by Zubay and Morowitz.

Cairns-Smith A.G- 1982-<u>Genetic takeover and the mineral origins of life</u>. 425 pgs. Cambridge University Press, Cambridge, U.K..

deDuve, C- 1995-<u>Vital Dust: Life as a cosmic imperative</u>. 302 pgs. Basic Books, N.Y., N.Y.

Dyson F- 1999-<u>Origins of Life</u>- 100 pgs. 2nd edition. Cambridge University Press, Cambridge, U.K.

Fry I- 2000-<u>The Emergence of Life on Earth: A Historical and Scientific overview</u>- 283 pgs. Rutgers U. Press, New Brunswick, N.J.

Morowitz H.J- 1992-<u>Beginnings of Cellular life. Metabolism recapitulates biogenesis</u>. 178 pgs. Yale University Press, New Haven, Conn.

Strick J.E. 2000-<u>Sparks of Life. Darwinism and the Victorian debates over spontaneous generation</u>. 201 pages. Harvard University Press, Cambridge, MA.

Zubay G- 2000-<u>Origins of Life on the Earth and in the Cosmos</u>. 2nd edition. 509 pgs. Academic Press, San Diego, CA.

We tend to think of **catalysis** as requiring huge and complex molecules. These articles note how several simple catalysts, working together, can catalyze reactions in a fairly specific manner.

Schreiner P.R- 2010-Cooperativity tames reactive catalysts- Science 327: (#5968, 2/19) 965-966

Xu H., S.J. Zuend, M.G. Woll, Y. Tao, E.N. Jacobsen- 2010- Asymmetric cooperative catalysis of strong Brønsted acid-promoted reactions using chiral ureas- Science 327: (#5968, 2/19) 986-990

The geologists can tell from minerals whether life was present or not. Here is an article that addresses the **geological state** of the **early earth**, and another that describes how life has altered minerals on this planet over time.

Hazen R.M- 2010-Evolution of minerals- Scientific American 302: (#3, March) 58-65

Simpson S- 2010-Violent origins of continents- Scientific American 302: (#1, Jan.) 60-67

The condition of the early atmosphere that might have been present on the early earth has recently been reconsidered.

Chyba C.F- 2010-Countering the early faint sun- Science 328: (#5983, 6/4) 1238-1239

Wolf E.T., O.B. Toon- 2010-Fractal organic hazes provided an ultraviolet shield for early earth- Science 328: (#8983, 6/4) 1266-1268

The role of membranes and lipids in the origin of life has inspired some to call for a **"lipid world"** view of early proto-life structures.

Segré D., D. Ben-Eli, D.W. Deamer, D. Lancet- 2001-The lipid world- Origins of life and evolution of the biosphere 31: (#1-2) 119-145
Walde P- 2006-Surfactant assemblies and their various possible roles for the origin(s) of life- Origins of life and evolution of biospheres- 36: 109-150

Early **genetic material** is often put in the context of the **RNA world** here are some article about this topic. But be aware that some have proposed other compounds that could have served as genetic material before RNA, some other **self-replicating systems** are noted in the articles below..

Bean H.D., F.A.L. Anet, I.R. Gold, N.V. Hud- 2006-Glyoxylate as a backbone linkage for a prebiotic ancestor of RNA- Origins of life and evolution of biospheres- 36: 39-63
Carnall J.M.A., C.A. Waudby, A.M. Belengner, M.C.A. Stuart, J.J-P. Peyralans, S. Otto- 2010-Mechanosensitive self-replication driven by self-organization- Science 327: (#5972, 3/19) 1502-1506
Franchi M., E. Gallori- 2004-Origin, persistence and biological activity of genetic material in prebiotic habitats- Origins of Life and Evolution of the Biosphere 34: 133-141
Joyce G.F- 1991-The rise and fall of the RNA world- The New Biologist 3: 399-407
Lincoln T.A., G.F. Joyce- 2009-Self-sustained replication of an RNA enzyme- Science 323: (#5918, 2/27) 1229-1232
Nagaswamy U., G.E. Fox- 2003-RNA ligation and the origin of tRNA- Origins of life and evolution of the biosphere- 33: 199-209
Robertson M.P., W.G. Scott- 2007-The structural basis of ribozyme-catalyzed RNA assembly- Science 315: (3/16) 1549-1553
Santoro S.W., G.F. Joyce- 1997-A general purpose RNA-cleaving DNA enzyme- Proceedings of the National Academy of Science (USA) 94: 4262-4266
Schechner D.M., R.A. Grant, S.C. Bagby, Y. Kolodobskaya, J.A. Piccirilli, D.P. Bartel- 2009-Crystal structure of the catalytic core of an RNA-polymerase ribozyme- Science 326: (#5957, 11/27) 1271-1275
Sowerby S.J., P.A. Stockwell, W.M. Heckl, G.B. Petersen- 2000-Self-programmable, self-assembling two-dimensional genetic matter- Origins of life and evolution of the biosphere 30: 81-99
Tjivikua T., P. Ballester, J. Rebek jr- 1990-A self-replicating system- Journal of the American Chemical Society 112: 1249-1250
Wilson T.J., D.M.J. Lilley- 2009-The evolution of ribozyme chemistry- Science 323: (#5920, 3/13) 1436-1438

The Martian meteorite ALH84001 turns out not to have microfossils of life. But it does have prebiotic nanostructures, and similar structures are found in life. This may be another way to form **microcompartments** in early **pre-biotic forms**.

Young J.D., J. Martel- 2010-The rise and fall of nanobacteria- Scientific American 302: (#1, Jan.) 52-59

Here are some studies examining how various types of **organic molecules** can be formed abiotically, on Earth and elsewhere.

Atreya S- 2007-Titan's organic factory- Science 316: (5/11) 843-845

Chyba C., C. Sagan- 1992-Endogenous production, exogenous delivery and impact-shock synthesis of organic molecules: An inventory for the origins of life- Nature 355: 125-132

Johnson A.P., H.J. Cleaves, J.P. Dworkin, D.P. Glavin, A. Lazcano, J.L. Bada- 2008-The Miller volcanic spark discharge experiment- Science 322: (#5900, 10/17) 404

McCollom T.M., G. Ritter, B.R.T. Simoneit- 1999-Lipid synthesis under hydrothermal conditions by Fischer-Tropsch-type reactions- Origins of life and evolution of the biosphere 29: 153-166

Lambert J.B., S.A. Gurusamy-Thangavelu, K. Ma- 2010-The silicate-mediated formose reaction: Bottom-up synthesis of sugar silicates- Science 327: (#5968, 2/19) 984-986

Miller S.L- 1953-A production of amino acids under possible primitive earth conditions- Science 117: 528-529

Miller S.L., H.C. Urey- 1959-Organic compound synthesis on the primitive Earth- Science 130: 245-251

Proskurowski G., M.D. Lilley, J.S. Seewald, G.L. Früch-Green, E.J. Olson, J.E. Lupton, S.P. Sylva, D.S. Kelley- 2008-Abiogenic hydrocarbon production at lost city hydrothermal field- Science 319: (#5863, 2/1) 604-607

Reinmann R., G. Zubay- 1999-A feasible step in the prebiotic pathway for RNA- Origins of life and evolution of the biosphere 29: (#3) 229-247

Tsukahara H., E-I. Ima, H. Honda, K. Hator, K. Matsuno- 2002-Prebiotic oligomerization on or inside lipid vesicles in hydrothermal environments- Origins of life and evolution of the biosphere 32: 13-21

Weber A.L- 2001-The sugar model: Catalysis by amines and amino acid products- Origins of life and evolution of the biosphere 31: 71-86

This article describes how **membranes** could form spontaneously.

Percec V., D.A. Wilson, P. Leowanawat, C.J. Wilson, A.D. Hughes, M.S. Kaucher, D.A. Hammer, D.H. Levine, A.J. Kim, F.S. Bates, K.P.Davis, T.P. Lodge, M.L. Klein, R.H. DeVane, E. Aqad, B.M. Rosen, A.O. Argintaru, M.J. Sienkowska, K. Rissanen, S. Nummelin, J. Ropponen- 2010-Self-assembly of janus dendrimers into uniform dendrimersomes and other complex architectures- Science 328: (#5981, 5/21) 1009-1014

Simple organic molecules have been shown to form complex structures, some of which might serve as **microcompartments**.

Stefankiewicz A.R., J.K.M. Sanders- 2010-Harmony of the self-assembled spheres- Science 328: (#5982, 5/28) 1115-1116

Sun Q-F., J. Iwasa, D. Ogawa, Y. Ishido, S. Sato, T. Ozeki, Y. Sei, K. Yamaguchi, M. Fujita- 2010- Self-assembled $M_{24}L_{48}$ polyhedra and their sharp structural switch upon subtle ligand variation- Science 328: (#5982, 5/28) 1144-1147

Looking for a new home? One of the articles below will help you identify **galactic habitable zones**. Also, it helps to find a world with a strong **magnetic field** to protect from loss of the atmosphere. And, you've seen it in movies; but how likely is it that life came to earth from space? Some studies of the survival of **life in space** have been done.

Gonzalez G- 2005-Habitable zones in the universe- Origins of life and evolution of biospheres 35: 555-606

Green R.H., D.M. Taylor, E.A. Gustan, S.J. Fraser, R.L. Olson- 1971-Survival of microorganisms in a simulated martian environment- Space Life Sciences 3: 12-14

Jardine M- 2010-Sunscreen for the young Earth- Science 327: (#5970, 3/5) 1206-1207

Tarduno J.A., R.D. Cottrell, M.K. Watkeys, A. Hofmann, P.V. Doubrovine, E.E. Manajek, D. Liu, D.G. Sibeck, L.P. Neukrich, Y. Usui- 2010-Geodynamo solar wind, and magnetopause 3.4-3.45 billion years ago- Science 327: (#5970, 3/5) 1238-1240

Weiss B.P, J.L. Kirschvink- 2000-Life from space? Testing panspermia with Martian meteorite ALH84001- The Planetary Report 20: (#6, Nov/Dec) 8-11

BIO 108   2010
Day 13, Lecture 32, Title: Ecology, abiotic factors.
Text Readings: Campbell et al. (2008), chapter 52, and pgs. 1226-1228.

Topics to cover:

> **Abiotic Factors**
> > **Light Intensity**
> > **Temperature and Air Flow (and water flow)**
> > **Seasonal and Geographic Factors**
> > **Mineral Limitations**
> **Biomes**
> > **Aquatic**
> > **Tundra**
> > **Desert**
> > **Savanna**
> > **Rainforest**
> **Summary**

Abiotic Factors
Ecosystems include not just the community of many species in an area, also includes the abiotic factors. These factors often greatly influence the nature of the ecosystem...
Abiotic factors that influence biomes include:
> 1.) Light intensity
> 2.) Air flow and temperature
> 3.) Seasonal and geographic factors
> 4.) Mineral availability
Can we make preditions of the type of ecosystem from abiotic factors?
> Example, use of a climograph, plot of mean percipitation versus mean temperature
> Fig. 52.20, temp. vs. rainfall for biomes
> Even with no other information could tell something about a region from just two factors
> However, averages over a year do not tell whole story...
Want to focus on those big issues that would be true even if we went to another world.
Will then relate this to some representative biomes on our planet.

> **Light Intensity**
> Fig. 52.10, solar radiation and latitudes
> Due to curvature of earth same solar energy is spread out over broader area at poles
> This alters the energy delivered per area, changing the temperature achieved
> Energy delivered is not greatly influenced by distance of planet from the sun, or by
> > intensity of sun, these do not change much for earth, and are not seasonal
> thickness and nature of atmosphere, has some influence here...
> > changes in reflection from the clouds alters the albedo of the planet

Temperature and Air Flow (and water flow)
Light intensity differences per area creates temperature gradients, leading to air flow
    Fig. 52.10, global air circulation
Warm air is less dense (low air pressure), and can hold more water in it as water vapor
    As it rises it comes under lower pressure, so it expands and cools
    cooler air can not hold as much water vapor so clouds form and there are storms
    so at such regions have high surface temperatures, and lots of rain
Rising warm air also pulls air in laterally
    and over certain distances have a convection cell created
Falling air warms as it falls (higher air pressure), and so is dry, its water holding ability rises
    On Earth this produces deserts at +/- 30 degrees latitude
This may account for banding of cloud patterns on other worlds?
    Pg. 163, (Sagan, 1980) Saturn.jpg
        a computer graphic, based on Pioneer 11 images note banding patterns
Add in rotation of Earth, and this generates the trade winds
        Fig. 52.10, trade winds
    Not perfect as land/water differ in albido
    So can find the deserts vs. areas of high rain
        Fig. 50.19, major biomes in world
It can also explain shore breezes
    light on land warms it, by afternoon the air over land rises,
    this pulls air from off cooler nearby lake or ocean, makes a shore breeze
    Fig. 52.12, shore breeze
Water flow and temperature differences
        Fig. 3.5 (Molles 2008) ocean circulation.jpg
    Can make analogous argument for water circulation in oceans,
        but also pushed by winds, water pushed from a coast pulls water up
        Note wind blows away from N. Africa, and pushes water away as well.
    Of course the wind moves the surface waters, deep waters also move
        Fig. 52.11, deep water circulation
        This brings oxygenated water to deep areas, distributes heat, and
        brings minerals to surface where theser is upwelling.
    So such flow greatly affects aquatic biomes
    Note the gulf stream is one part of the above
    This brings warmer water north
    based on density differences of water, so both saltiness and winds play a role...
    Can also change water density by changes in saltiness, via melting freshwater ice?

Seasonal and Geographic Factors
The average temperature, rainfall, or day length does not tell the whole story.
Need to know the extent of variation as well. Much of this can be due to tilt of the earth
        Fig. 52.10, tilt of earth and seasons
    Fairly steady sunlight at equator, due to tilt the poles change alot

Extreme changes at poles: total darkness to total sunlight over 24 hours.
Fig. 14.8, (Barbour etal, 1987) daily_solar_radiation.jpg
note that in summer can get 24 hour a day insolation at poles
can get more light energy at poles per day than at equator, but briefly
at poles in winter see no sunlight. So poles show the most change over the year.
Coastal versus continental temperature variations on land
Fig. 14.10, (Barbour etal, 1987) temperature_variation.jpg
Oceans buffer temperature swings
Greater variation in middle of continents
Rainshadow effect
Fig. 52.13, rainshadow effect
air rises as crosses mountains, rising air cools, get rain, and rain shadow
as air past the moutains holds less water and falls and warms
Fig. 52.10, (again) global wind patterns
Depending on direction of winds will get rainshadow on one side or other
Fig. 52.19, terrestrial biomes
Note effects of mountain ranges: Sierra Nevada mountains, Andes Mountains
So if we have the geography of a world mapped, know its tilt, and air circulation cells, we
should be able to determine seasonal and geographical rainfall patterns

Mineral Limitations
Often mineral availability can limit productivity of a biome.
by productivity mean net photosynthetic activity.... or other measures....
Minerals can be limiting on land due to
excess rain washes out minerals
local rocks lack proper minerals
minerals are locked up by living organisms, this is biotic competition
minerals are trapped in dead organisms that are not easily rotted
Or minerals can be present on land that are toxic
some regions of Brazil have high [Al] in soil
only certain types of plants can tolerate this
Minerals in aquatic ecosystems can be limiting as well
dead organisms may fall to bottom and carry minerals with them
making the surface waters nutrient poor...

Biomes
Example biomes: Now want to consider biomes in terms of these abiotic factors.

Aquatic
Consider a freshwater Lake: density of water, good thing ice is not most dense!
Fig. 50.13, (Campbell and Reece, 2005) lake stratification
Note distribution of oxygen as go further down into lake,
bottom will be cold and may go anaerobic

Nutrients trapped in lower water, but often low oxygen gas concentration as well..
    so this can limit rates of metabolism even with lots of food present?
At spring and fall have same temperature vertically,
    so water turns over, nutrients brought to surface
    oxygen brought to bottom, this mixing produces algal blooms
    this shows the limitation of minerals, so at other times there is low productivity
Consider two examples of extremes in lakes (many intermediate states exist...)
    Fig. 52.18, oligotrophic lake and eutrophic lake
    Oligotrophic lakes: low in nutrients, low algal growth, a lake with clear waters
    Eutrophic lakes: higher nutrients, higher algal growth, often bottom oxygen low

Now consider the situation in the Oceans:
    Places where nutrients up-well in ocean are higher in productivity
        up welling is where waters from depths come to surface
            note again the north west coast of Africa...
            Fig. 55.6, net primary productivity of earth
        oceans are often nutrient poor, note most production near shore, river outflow...
        algae die, sink to bottom of ocean
            Fig. 52.16, zonation in marine environment
        just like in freshwater lakes
        major abiotic factors here are:
            Light, temperature, nutrients, and oxygen concentration
    Ocean biomes vary with changes in these factors.
            Fig. 52.15, major aquatic biomes
        most of ocean is very low in production, one exception is coral reefs
        Limitations often due to lack of iron and phosphates
        Coastal areas, such as marshes and mangroove swamps, are higher in nutrients,
            so more algae, and more plants, higher productivity....
    Evidence of nutrient limits in the ocean, seen in iron fertilization experiments
            Table 55.1, nutrient supplimentation
        led to more nitrogen fixation, more photosynthesis, short term rise in productivity.
    Nice article, Falkowski (2002), that reviews phytoplankton growth and ocean minerals.
        Argues that oceans are often iron and micronutrient poor. These algae cycle
            rapidly in numbers and might bind up much carbon dioxide.
        Each year phytoplankton fixes 45-50 billion metric tons of carbon. This is 2X
            higher than earlier estimates. Land plants fix about 52 billion metric tons
            of C each year, a much lower estimate than previously thought.
            Phytoplankton can grow fast enough to replace all of its biomass in one
            week, while land plants would have to grow 20 years to replace its
            biomass.

Fixed carbon at surface of ocean is warm, has oxygen available, so is often rapidly respired. At > 200 m of depth in ocean it is cooler and lower oxygen is available, so carbon compounds getting there will last longer. Estimates 7-8 billion metric tons of fixed C from phytoplankton can go deep in ocean each year. This pulls carbon dioxide out of the atmosphere and lowers the carbon dioxide contentration in the atmosphere by about 200 ppm lower, some estimate, than it would otherwise be.

Could fertilizing upper oceans induce a stronger carbon sink and so control greenhouse effect?

Phytoplankton growth is limited by nitrogen. Iron is needed for nitrogen fixation. Iron can also be a limiting micronutrient. Ancient ice cores suggest that past rise in ocean [Fe] correlates with lower [CO_2] in atmosphere, and with ice ages. Much Fe gets to ocean in dust blown off of land.

Several companies have obtained patents on ocean fertilizing procedures, other patents applied for, and they plan to do massive fertilizing of open ocean and so promote carbon sink. And to sell the carbon credits...

How above will alter ocean ecosystem is not clear. Higher carbon sink might lower oxygen concentrations in deep ocean areas? Some argue that this is how the "dead zones" in ocean off of NYC and in Gulf of Mexico were formed. By nutrients coming down in rivers out to ocean.

Now consider terrestrial abiotic issues for specific biomes

Tundra

cold, so temperature is often limiting. Also sunlight is limiting half of the year

plants need shelter from wind, and water may be frozen and limiting as well

Fig. 52.19, major terrestrial biomes

Fig. 52.21, tundra

Variation is very high, recall light energy per day is higher in summer than in the tropics!

summer see rapid growth of plants, insects, etc

so birds migrate here... Caribou move over large areas for food

Fig. 50.20, (Campbell and Reece, 2005) tundra

they migrate in seasonally when local productivity is high...

Desert

Water is limiting, can be warm or cold desert

see producers good at going dormant, CAM plants reduce water loss...

Fig. 52.19, major terrestrial biomes

Fig. 50.20, desert collage

Savanna

has water but not evenly through out the year

       high season variation in rainfall, rain seasons and dry seasons....

       so plant growth is also seasonally variable

       Fig. 52.19, major terrestrial biomes

       Fig. 52.21, savanna

       often periodic fires due to dry seasons, removes many plants

Rainforest

here abiotic factors tend not to be limiting

but for many plants there are often nutrient limits, due to biotic competition

       dead plants release nutrients as they rot

       but these nutrients are quickly taken up by other plants and locked up

Also, high flow of water will leech out many of the free nutrients in the soil

so tropical soils tend to be very nutrient poor.

       Fig. 50.19, major terrestrial biomes

       Fig. 50.20, (Campbell and Reece, 2005) lowland rainforest

So soils are not holding the nutrients as much as the living organisms are.

       that is why loss of standing trees in rainforests leads to very poor soils

Some exceptions to the above, for instance

       Epiphytes, plants growing on other plants can be water and nutrient limited

           the tree trunks they grow on are nutrient and water deserts?

Summary

Fig. 52.20, climograph of biomes

Abiotic factors matter greatly in determining the productivity of ecosystems. Have considered

       light, temperature, moisture, minerals, geographical features...

       they interact greatly... and determine the type of species that are present.

Objectives:

How does the distribution of solar radiation differ across the Earth's surface, what causes this distribution, and what consequence does this have for temperature and regional climate? How does this influence the distribution of rainfall and of the trade winds?

How does the movement of water in the oceans act in a manner analogous to that of the movement of air? How is the flow of the gulf stream powered? What powers the movement of the air to create the trade winds?

How do geographic factors alter rainfall and regional temperatures? Describe the rainshadow effect, and if given a map showing geographic features and prevailing winds be able to identify areas of higher and lower rainfall.

In what way does the fact that the Earth is tilted contribute to the variation in climate? What role does the variation in the local climate play in determining the strategies used by species that attempt to survive in an area?

Describe how minerals can be locked up in a lake. What keeps the nutrient-rich water in the bottom of a lake from mixing well with the surface water? How is this similar to nutrient conditions in some parts of the ocean? How does this compare to how minerals are often locked up in tropical rainforests?

Be able to describe the following biomes, which are presented in figure 52.21, in terms of abiotic factors that have a major influence on each of them: Tropical forest, desert, savanna, coniferous forest, temperate broadleaf forest, and tundra.

For review, see self-quiz questions #2-4, 6, 8-9, and 12 of chapter 52.

Needed overheads and items:

Fig. 52.20, temp. vs. rainfall for biomes
Fig. 52.10, solar radiation and latitudes
Fig. 52.10, global air circulation
Pg. 163, (Sagan, 1980) Saturn.jpg
Fig. 52.10, tradewinds
Fig. 52.19, major biomes in world
Fig. 52.12, shore breeze
Fig. 3.5 (Molles 2008) ocean circulation.jpg
Fig. 52.11, deep water circulation
Fig. 52.10, tilt of earth and seasons
Fig. 14.8, (Barbour etal, 1987) daily_solar_radiation.jpg
Fig. 14.10, (Barbour etal, 1987) temperature_variation.jpg
Fig. 52.13, rainshadow effect
Fig. 52.10, global wind patterns
Fig. 52.19, major terrestrial biomes
Fig. 50.13, (Campbell and Reece, 2005) lake stratification
Fig. 52.18, oligotrophic lake and eutrophic lake
Fig. 55.6, net primary productivity of Earth
Fig. 52.16 zonation in marine environment
Fig. 52.15, major aquatic biomes
Table 55.1, nutrient supplimentation
Fig. 52.19, major terrestrial biomes
Fig. 52.21, tundra
Fig. 50.20, (Campbell and Reece, 2005) tundra
Fig. 52.19, major terrestrial biomes
Fig. 52.21, desert collage
Fig. 52.19, major terrestrial biomes
Fig. 52.21, savanna
Fig. 52.19, major terrestrial biomes
Fig. 50.20, (Campbell and Reece, 2005) lowland rainforest
Fig. 52.20, climograph of biomes

Handout:
Handout - lecture 32.stm
Fig. 14.8, (Barbour etal, 1987) daily_solar_radiation.jpg
Fig. 14.10, (Barbour etal, 1987) temperature_variation.jpg

References:

Barbour M.G., J.H. Burk, W.D. Pitts- 1987-<u>Terrestrial Plant Ecology</u>. 2nd edition. Figures 14.8, 14.10. Benjamin/Cummings Publishing Co. Menlo Park, CA.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-An introduction to ecology and the biosphere. Chapter 52. Pages 1148-1173 and 1226-1228. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition. Figures 50.13, 50.20. Benjamin Cummings Press. San Francisco, CA.

Falkowski P.G- 2002-The ocean's invisible forest- Scientific American 287: (#2) 54-61

Molles M.C.jr- 2008-<u>Ecology. Concepts & applications</u>. Fourth edition. Fig. 3.5. McGraw Hill. Boston, MA.

Sagan C- 1980-<u>Cosmos</u>. Pg. 163. Random House. N.Y., N.Y.

Related issues:

Changes in temperature in the oceans, attributed to global warming, have been suggested to lead to a rise in the number of **algal blooms**, in the **rate of evaporation** from the ocean. It may also alter the delivery of nutrients to the oceans carried in **meltwater**.

Li W.K.W., F.A. McLaughlin, C. Lovejoy, E.C. Cormack- 2009-Smallest algae thrive as the arctic ocean freshens- Science 326: (#5952, 10/23) 539

Molles M.C.jr- 2008-Temperature relations. Chapter 4, pgs. 82-107. In, Ecology. Concepts & applications. Fourth edition. McGraw Hill. Boston, MA.

Pearl H.W., J. Huisman- 2008-Blooms like it hot- Science 320: (#5872, 4/4) 57-58

Smetacek V., J.E. Cloern- 2008-On phytoplankton trends- Science 319: (#5868, 3/7) 1346-1348

Wentz F.J., L. Ricciardulli, K. Hilburn, C. Mears- 2007-How much more rain will global warming bring?- Science 317: (#5835, 7/13) 233-235

These articles describe attempts to understand how the **ocean currents** help to redistribute **heat**, and whether or not they are changing.

Church J.A- 2007-A change in circulation?- Science 317: (#5840, 8/17) 908-909

Cunningham S.A., T. Kanzow, D. Rayner, M.O. Baringer, W.E. Johns, J. Marotzke, H.R. Longworth, E.M. Grant, J.J-M. Hirschi, L.M. Beal, C.S. Meinen, H.L. Bryden- 2007-Temporal variability of the Atlantic meridional overturning circulation at 26.5° N- Science 317: (#5840, 8/17) 935-938

Kanzow T., S.A. Cunningham, D. Rayner, J.J-M. Hirschi, W.E. Johns, M.O. Baringer, H.L. Bryden, L.M. Beal, C.S. Meinen, J. Marotzke- 2007-Observed flow compensation associated witht he MOC at 26.5° N in the Atlantic- Science 317: (#5840, 8/17) 938-941

Lozier M.S., S. Leadbetter, R.G. Williams, V. Roussenov, M.S.C. Reed, N.J. Moore- 2008-The spatial pattern and mechanisms of heat-content change in the North Atlantic- Science 319: (#5864, 2/8) 800-803

This article examines the **heat balance** of the **earth**. There is a significant amount of heat that seems to have gone missing!

Trenberth K.E., J.T. Fasullo- 2010-Tracking Earth's energy- Science 328: (#5976, 4/16) 316-317

For more on **aquatic biomes** see: Including areas in the ocean of very **low oxygen** content. The effect of nutrients from **icebergs** on the local ocean area. And what is found at **hydrothermal vents** and deep under the sea floor.

Chan F., J.A. Barth, J. Lubchenco, A. Kirincich, H. Weeks, W.T. Peterson, B.A. Menge- 2008-Emergence of anoxia in the California current large marine ecosystem- Science 319: (#5865, 2/15) 920

Fisher C.R., P. Girguis- 2007-A proteomic snapshot of life at a vent- Science 315: (1/12) 198-199

Molles M.C.jr- 2008-Life in water. Chapter 3, pgs. 47-80. In, Ecology. Concepts & applications. Fourth edition. McGraw Hill. Boston, MA.

Roussel E.G., M-A.C. Bonavita, J. Querellen, B.A. Cragg, G. Webster, D. Prieur, R.J. Parkes- 2008-Extending the sub-sea floor biosphere- Science 320: (#5879, 5/23) 1046

Smith jr K.L., B.H. Robison, J.J. Helly, R.S. Kaufmann, H.A. Ruhl, J.J. Shaw, B.S. Twing, M. Vernet- 2007-Free-drift icebergs: Hot spots of chemical and biological enrichment in the Weddell Sea- Science 317: (#5837, 7/27) 478-482

Here are some articles dealing with features of **tropical forest biomes**.

Gullison R.E., P.C. Frumhoff, J.G. Canadell, C.B. Field, D.C. Nepstad, K. Hayhoe, R. Avissan, L.M. Curran, P. Friedlingstein, C.D. Jones, C. Nobre- 2007-Tropical forests and climate policy- Science 316: (5/18) 985-986

Malhi Y., J.T. Roberts, R.A. Betts, T.J. Killeen, W. Li, C.A. Nobre- 2008-Climate change, deforestation, and the fate of the amazon- Science 319: (#5860, 1/11) 169-172

Saleska S.R., K. Didan, A.R. Hueto, H.R. de Rocha- 2007-Amazon forests green-up during 2005 drought- Science 318: (#5850, 10/26) 612

Watkins J.E. jr, M.K. Mack, S.S. Mulkey- 2007-Gametophytic ecology and demography of epiphytic and terrestrial tropical ferns- American Journal of Botany 94: (#4) 701-708

Ecologists would like to be able to model **seasonal variations** closely. For instance, being able to model the **monsoons** and surface **ocean surface temperature** changes would be of great value. There are also attempts to model the rains in **Africa**, and how they might change with global warming.

Cook E.R., K.J. Anchukaitis, B.M. Buckley, R.D. D'Arrigo, G.C. Jacoby, W.E. Wrigth- 2010-Asian monsoon failure and megadrought during the last millennium- Science 328: (#5977, 4/23) 486-489

Patt A.G., L. Ogallo, M. Hellmuth- 2007-Learning from 10 years of climate outlook forums in Africa- Science 318: (#5847, 10/5) 49-50

Shukla J- 2007-Monsoon mysteries- Science 318: (#5848, 10/12) 204-205

Smith D.M., S. Cusack, A.W. Colman, E.K. Folland, G.R. Harris, J.M. Murphy- 2007- Improved surface temperature prediction for the coming decade from a global climate model- Science 317: (#5839, 8/10) 796-799

Wahl E.R., C. Morrill- 2010-Toward understanding and predicting monsoon patterns- Science 328: (#5977, 4/23) 437-438

This study attempts to follow the circulation of the **atmosphere** across the globe to estimat how long **air pollution** can last and how it spreads..

Randel W.J., M. Park, L. Emmons, D. Kinnison, P. Bernath, K.A. Walker, C. Boone, H. Pumphrey- 2010-Asian monsoon transport of pollution to the stratosphere- Science 328: (#5978, 4/30) 611-613

Here are some articles that examine aspects of **semi-arid regions** that are between savannah and desert. The affects of planting trees in such regions can include making things hotter!

Rotenberg E., D. Yakir- 2010-Contribution of semi-arid forests to the climate system- Science 327: (#5964, 1/22) 451-454

Schimel D.S- 2010-Drylands in the Earth system- Science 327: (#5964, 1/22) 418-419

Another biome on Earth is the **crustal biome**, which extends kilometers down from the surface. Various prokaryotes have been found to live that far down!

Lin L-H., P-L. Wang, D. Rumble, J. Lippmaan-Pipke, E. Boice, L.M. Pratt, B.S. Lollar, E.L. Brodie, T.C. Hazen, G.L. Andersen, T.Z. DeSantis, D.P. Moser, D. Kershaw, T.C. Onstott- 2006-Long-term sustainablity of a high-energy, low-diversity crustal biome- Science 314: (10/20) 479-482

Newman D.K- 2010-Feasting on minerals- Science 327: (#5967, 2/12) 793-794

Here are studies on **oceanic nutrient supplimentation** and studies of how **ocean acidification** may alter the availability of marine nutrients including **iron**.:

Basu S- 2007-Ocean going iron- Scientific American 297: (#4, Oct.) 23-24

Buesseler K.O., S.C. Doney, D.M. Karl, P.W. Boyd, K. Caldeira, F. Chai, K.H. Coale, H.J.W. deBaar, P.G. Falkowski, K.S. Smetacek, S. Takeda, A.J. Waston- 2008- Ocean iron fertilization - moving forward in a sea of uncertainty- Science 319: (#5860, 1/11) 162

Conley D.J., H.W. Paerl, R.W. Howarth, D.F. Boesch, S.P. Seitzinger, K.E. Havens, C. Lancelot, G.E. Likens- 2009-Controlling eutrophication: Nitrogen and phosporus- Science 323: (#5917, 2/20) 1014-1015

Kintisch E- 2007-Should oceanographers pump iron?- Science 318: (#5855, 11/30) 1368-1370

Shi D., Y. Xu, B.M. Hopkinson, F.M.M. Morel- 2010-Effect of ocean acidification on iron availability to marine phytoplankton- Science 327: (#5966, 2/5) 676-679

Sunda W.G- 2010-Iron and carbon pump- Science 327: (#5966, 2/5) 654-655

BIO 108   2010
Day 13, Lecture 33, Title: Population Ecology.
Text Readings: Campbell et al. (2008), Chapter 53.

Topics to cover:

> **Characterizing Populations**
> **Species Strategies influence their population characteristics**
> **Population Growth Equations**
> **Population Structure**
> **Reproductive Strategies**
> **Population Genetics**
> **Summary**

Characterizing Populations
Populations make up the biological communities in an ecosystem.
> A population includes all the individuals of the same species in an area
Many interesting questions about populations:
> How large is it?
> What is the range of this population over time? What physical areas does it cover?
> Is the population stable? Is it evolving? How genetically diverse is it?
> What selective pressures does it experience?
> Who are the reproductively important individuals?
> What is the age structure of the population?
These questions are interesting and important for conservation efforts.
How to determine Population size and distribution?
> A species characterized by scattered individuals
> > differs from one that exists in clumps or packs
> Other different strategies, and issues
> > Density-dependent factors, such as disease, local food supply, etc...
> > > may be specific to a species?
> > Density-independent factors, such as weather, can also limit range
> Sampling of an area, then have to extrapolate across range, with obvious assumptions...
> Mark and recapture methods
> > Example: Catch 100 individuals, mark them, Release
> > Allow time for redistribution,
> > > but not enough for significant chance of death
> > > Assume the animal does not learn to avoid trap, etc...
> > > Also assume that marking does not alter its chance to survive and mate
> > After time, capture another 100 individuals, 20 of them have marks on them

Then work a proportion, as given in the book:

$$\frac{\text{# of recaught in second catch}}{\text{Total # in second catch}} = \frac{\text{# marked in first catch}}{\text{Total population #}}$$

With some algebra this becomes:

$$\frac{\text{# of recaught in second catch}}{\text{# marked in first catch}} = \frac{\text{Total # in second catch}}{\text{Total population #}}$$

example:

20 marked-recaught/100 total marked

= 100 second catch/total population

So total population estimated = 500

While doing this, can also note traits of individuals caught such as:

age, sex, take tissue samples, etc...

Species Strategies influence their population characteristics

Species strategies, see sets of traits that associate in certain patterns

These are trends, many exceptions. Will cover two such patterns

Some are masters of interactions: K-selected strategists

tend to occur in more stable environments

so steady selective pressures (Which land biome would this be?)

interactions are often very species-specific

populations tend to persist near carrying capacity

Others depend on disturbances and interact poorly: R-selected strategists

opportunists, might come in right after a major disturbance

fire, storm, etc

must disperse well to such areas

not as good at species-species interactions

populations tend to grow rapidly and then fall, boom-and-bust

Keep these patterns in the back of your mind as we as cover the following issues...

Population Growth Equations

Population growth is a trait of a species

how fast a population can grow has an upper limit characteristic of a species

What total population size can be achieved may depend on local resouces

Two major models: Exponential and Logistic growth. These are "ideal" cases...

Fig. 53.12, population growth

Exponential growth equation: $\Delta N / \Delta t = rN$

r = b - d = intrinsic growth rate, per captita,

(has units of: #/t/# = number per time per number)

b = birth rate per capita (#/t/#)

d = death rate per capita (#/t/#)

these differ with different species

N = initial population size (#)

$\Delta N / \Delta t$ = change in number per a given change in time (#/t)

Fig. 53.10, exponential growth
  Disturbance creates open niches
    fast population growth rates fills it
    disturbance is population independent
  This is a strategy for species coming in after disturbance.
    also see this in spread of many pathogens into new host populations
    species using this approach do not have
      to interact well with others
      a means to avoid predation?
    few limits on growth while doing exponential growth
      if there were, how could it grow exponentially?
    selection for high intrinsic growth rate
Example:
  Mosquito populations over the summer
  boom and bust, so typical of R-strategists
  winter as a disturbance (biotic or abiotic?)
  rapid population growth
  predators of the mosquitos come after the population has grown
  after a few weeks no longer exponential, but initally it is...
<u>Logistic growth equation</u>:
  Fig. 53.12, logistic growth
  $\Delta N/\Delta t = rN(K-N)/K$
    K = carrying capacity (#)
      varies from area to area and over time of year
      also species specific, elephants versus moles have different K values
    Density-dependent factors
    Fig. 52.15, (Campbell and Reece, 2005) loss of fecundity with density
      Fecundity is the reproductive potential (# of potential offspring).
        this alters birth rate, and so by extension the "r" value
      what is limiting the population growth, not all just K?
      Many limiting factors only play a role at high population densities
      When see this then ask what factor is the cause...
    This is a strategy for species in more
      stable area, producing more stable populations.
      This may involve interactions between species.
    K-selected does not mean it does not change in population size over time
      See several examples of this...
      Fig. 52.19x, (Campbell and Reece, 2005) crab population
      Fig. 53.20, bunnies and lynx population cycle
      Notice how predator and prey populations follow each other.

3

Population Structure
Two populations of similar sizes can still differ in population structure
Age distribution in the population is one way the population can be structured
        Fig. 53.25, age-structure pyramids
                even with same number of individuals
                        can have growth,slow growth, shrinkage
                So age distribution in population matters
                so does sexual distribution
        Who would get to reproductive age first: R-selected or K-selected?
So over time how will a population structure change?
Could get similar population sizes, or growth, in many ways
        Fig. 53.24, demographic shift
        Consider Mexico, high birth rate and low death rate, difference gives a positive r
        Then Sweden, even with smaller birth and higher death rates, the r value may be positive?
        Note lately Sweden is near zero population growth, while Mexico is not...
Human population growth rate
        Fig. 53.23, human population growth rate
        not at highest it could be, has dropped a bit lately, but it is still growing
        Is this due to more starvation and disease, or to human choice, or both?
This relates to issues of Suvivorship and Fecundity, two aspects of life history of a species
        Table 53.2, reproductive table for ground squirrels
                Note litter size, and age of maturity, how fecundity changes with age
        Table 53.1, life table of ground squirrels
        Note average life expectancy but some do live many years
                Difference between males and females
                Differences between young and old...
        This sort of information can help you analyze the species' strategies...
                (It is also the sort of analysis used by insurance companies...)
        Suvivorship curves
                Fig. 53.6, idealized suvivorship curves
                this can vary within a species as well...
                rate of mortality may be initially high or low
                age to initial reproduction may vary as well

Reproductive Strategies
How long do members of a species have to survive before they can reproduce?
        Fig. 53.7, century plant
        Semelparity, one period of reproduction, then dies
                in case of century plant, plant lives to old age
                can wait for good conditions for young...?
        Iteroparity, reproduces several times over a life.
                this can alter the number of offspring per parent
Which of these is most likley to be associated with R strategy, and which with K strategy?

Population Genetics
We have covered this in an earlier lecture
     Genetic diversity is another trait of a population
Consider sexual vs. asexually reproducing species
     dandelions reproduce asexually
          so not genetically diverse, live in same habitat... is this good for R or K strategy?

Summary
Many features of populations differ,
     and can be associated with major differences in species strategies
Think about how the options presented here fit into the two strategies presented...

Objectives:

Contrast R- and K-selected strategies in terms of not just population growth but also the characteristics of a species that uses each strategy. What features of the environments in which these species live may have resulted in the selection for either of these strategies?

Understand the meaning of the terms in the exponential and logistic growth equations. If given values for these terms be able to calculate an estimate of future population change.

What assumptions are made, and what would you wish to know, before attempting to do a mark and recapture study of an animal species? Be able to use sample data to estimate total population size.

How does the age structure of a population relate to the strategy a species adopts? How would the age structure differ between a population that is growing versus one that is shrinking? Does fecundity stay the same with age? What are semelparity and iteroparity, and how would a population's age structure likely differ for each of these cases?

What are some density-dependent limits on population growth? What advantages do individuals of a species gain from living in groups? What advantages do individuals gain from living in a more scattered pattern?

For review, see self-quiz questions #1-5, 7-9, of chapter 53.

Needed overheads and items:

Fig. 53.12, logistic growth
Fig. 53.10, exponential growth
Fig. 53.12, logistic growth
Fig. 52.15, (Campbell and Reece, 2005)) loss of fecundity with density
Fig. 52.19x, (Campbell and Reece, 2005) crab population
Fig. 53.20, bunnies and lynx population cycle
Fig. 53.25, age-structure pyramids
Fig. 53.24, demographic shift
Fig. 53.23, human population growth rate
Table 53.2, reproductive table for ground squirrels
Table 53.1, life table of ground squirrels
Fig. 53.6, idealized suvivorship curves
Fig. 53.7, Century plant

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Population ecology. Chapter 53. Pages 1174-1197. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition. Figures 52.15, 52.19x. Benjamin Cummings Press. San Francisco, CA.

Related issues:

Here are some articles that describe **population dynamics** and attempts to model population changes over time.

Erwin D.H- 2005-Seeds of diversity- Science 308: (6/17) 1752-1753

Reynolds J.D., R. P. Freckleton- 2005-Population dynamics: Growing to extremes- Science 309: (7/22) 567-568

Sibly R.M., D. Barker, M.C. Denham, J. Hone, M. Pagel- 2005-On the regulation of populations of mammals, birds, fish, and insects- Science 309: (7/22) 607-610

In some cases the release of individuals is used to try to maintain or **reintroduce** a population. This requires knowledge of the life stages of the species, and can introduce new vulnerabilities. For instance, introducing salmon has also introduced a new parasite. Or, introducing tigers might alter the population genetics of the population, or using **captive breeding** to maintain pandas, and other species such as wolves. And attempts to restore the american chestnut.

Enserink M., G. Vogel- 2006-The carnivore come back- Science 314: (11/3) 746-749

Guo J- 2007-Giant panda numbers are surging - or are they?- Science 316: (5/18) 974-975

Krkošek M., J.S. Ford, A. Morton, S. Lele, R.A. Myers, M.A. Lewis- 2007-Declining wild salmon populations in relation to parasites from farm salmon- Science 318: (#5857, 12/14) 1772-1775

Miller G- 2010-In central California, Coho salmon are on the brink- Science 327: (#5965, 1/29) 512

Morell V- 2008-Wolves at the door of a more dangerous world- Science 319: (#5865, 2/15) 890-892

Morell V- 2007-Can the wild tiger survive?- Science 317: (#5843, 9/7) 1312-1314

Stilwell K.L., H.M. Wilbur, C.R. Werth, D.R. Taylor- 2003-Heterozygote advantage in the american chestnut, *Castanea dentata* (Fagaceae)- American Journal of Botany 90: (#2) 207-213

Stokstad E- 2007-Parasites from fish farms driving wild salmon to extinction- Science 318: (#5857, 12/14) 1711

One **reproductive strategy** is to have so many offspring as to overload the predators. Here is an article that relates to this approach.

Boutin S., L.A. Wanters, A.G. McAdam, M.M. Humphries, G. Tosi, A.A. Dhondt- 2006-Anticipatory reproduction and population growth in seed predators- Science 314: (12/22) 1928-1930

Winn A.A., P.A. Werner- 1987-Regulation of seed yield within and among population of *Prunella vulgaris*- Ecology 68: (#5) 1224-1233

Here is a review that has cautions about the uses of a species' **life history information** and how to compare it to other species.

DeJong G- 2005-Is invariance across animal species just an illusion?- Science 309: (8/19) 1193-1195

These papers examine **density dependent effects** on individuals in a population.

Eldakar O.T., M.J. Dlugos, J.W. Pepper, D.S. Wilson- 2009-Population structure mediates sexual conflict in water striders- Science 326: (#5954, 11/6) 816

Miller T.E., A.A. Winn, D. W. Schemske- 1994-The effects of density and spatial distribution on selection for emergence time in *Prunella vulgaris* (Lamiaceae)- American Journal of Botany 81: (#1) 1-6

Reynolds J.D., R. P. Freckleton- 2005-Population dynamics: Growing to extremes- Science 309: (7/22) 567-568

Monitoring population sizes require ongoing work. In some cases new data may inform debates about how to manage harvesting of animal populations. Here are some examples relating to commercial fishing. Also some examples are given below dealing with elephants, polar bears, eagles, sharks and saola populations.

Almany G.R., M.L. Berumen, S.R. Thorrold, S. Planes, G.P. Jones- 2007-Local replenishment of coral reef fish populations in a marine reserve- Science 316: (5/4) 742-744

Fryxell J.M., C. Packer, K. McCann, E.J. Solberg, B-E. Saether- 2010-Resource management cycles and the sustainability of harvested wildlife populations- Science 328: (#5980, 5/14) 903-906

Koenig R- 2007-Researchers explore alternatives to elephant culling- Science 315: (3/9) 1349

Morell V- 2007-Into the deep: First glimpse of Bering Sea canyons heats up fisheries battle- Science 318: (#5848, 10/12) 181-182

Pala C- 2007-Conservationists and fishers face off over Hawaii's marine riches- Science 317: (#5836, 7/20) 306-307

Pauly D., R. Watson- 2003-Counting the last fish- Scientific American 289: (#1) 42-47

Pennisi E- 2007-U.S. weighs protection for polar bears- Science 315: (1/5) 25

Skewagar E., P.D. Boersma, G. Harris, G. Caille- Anchovy fishery threat to Patagonian ecosystem- Science 315: (1/5) 45

Stokstad E- 2007-Can the bald eagle still soar after it is delisted?- Science 316: (#5832, 6/22) 1689-1699

Stone R- 2007-The last of the leviathans- Science 316: (#5832, 6/22) 1684-1688

Stone R- 2006-The Saola's last stand- Science 314: (12/1) 1380-1383

This article describes how a change in climate, and so in food supply, has threatened a population of **penguins**.

Stokstad E- 2007-Boom and bust in a polar hot zone- Science 315: (3/16) 1522-1523

This article examines how **sex bias** of offspring can occur in a population.

Cox R.M., R. Calsbeek- 2010-Cryptic sex-ratio bias provides indirect genetic benefits despite sexual conflict- Science 328: (#5974, 4/2) 92-94

BIO 108   2010
Day 14, Lecture 34, Title: Community Ecology.
Text Readings: Campbell et al. (2008), Chapter 54.

Topics to cover:

      What is a Community?
          Role of disturbances
          Individualistic vs. interactive hypotheses
          Types of species-species interactions
      Coevolution and Invasive Species
      Keystone Species
      Species Richness and Species Area curves
      Summary

What is a Community?
An assemblage of species that is often repeated and seen in areas that share certain environmental
      factors and biotic interactions over time...
These species have biotic interactions with each other, and abiotic interactions with area
      we have covered abiotic factors before, here will touch here mainly on biotic ones...
          species can alter their habitat, and these changes can affect other species
      these interactions are part of what define a community, and what interest us greatly
Is a community a chance event or is it repeatable? How does it change over time?
      Example of glacial retreat in Alaska
          Fig. 54.22, glacial retreat in Alaska
      If want longer time frame can look at
          glacial retreat over past 12000 yrs from here?
      Can go up several transects and repeat observations
          if community is repeatable should see similar changes over time?
      Primary succession, starts on bare rock with no soil, or where soil removed
          Such as where glacier retreats
      Pioneering species here include mosses, fireweed, etc
          Fig. 54.22, fireweed stage
      Alder trees come in next, associates with N-fixing bacteria
          Fig. 54.22, alder stage
      Better soils promotes spruce, hemlock to enter into area
          Fig. 54.22, hemlock and spruce
      But rich organic matter and shallow ponds promotes moss bogs
          moss acidifies the waters, alters nutrient levels
             moss sucks up nutrients better than spruce
          a nice example of a species altering its habitat?
      Table 53.2, (Campbell and Reece, 2002) Succession in Glacier Bay
      So at this level there is something repeatable.

Succession, community changes seen over time, often in repeatable ways
    here see a pattern for the retreat of this glacier
    This pattern is in part due to species affects on the area
        biotic interactions help determine what next species can come into area

Role of disturbances
The glacier was a major disturbance to area, other disturbances occur
Disturbance's role, abiotic disturbances
    Fire in grassland and forest, fire is a major disturbance
        Fig. 53.21, (Campbell and Reece, 2005) grassland fires
        Fig. 54.21, recovery after fire
    To conserve species in the grassland, may need periodic fires
        to give certain flowering plants a chance to reproduce need fires
        so some fires are an opportunity for some species
    Recovery from strong disturbances are often primary succession, this is secondary
Biotic disturbances, Facilitator species
    alter the local conditions and so which other species are present
        saw this done by several species with succession in glacial retreat?
    Beavers alter local conditions
        Fig. 54.17, beavers
        make ponds, flood areas, feed on local trees
        so alters species composition by changing the environment itself..
    rushes interact with other species and alter local community
        Fig. 54.18, black rush
        note differences here with versus without rushes in area
        Rush alters the soil chemistry,
            this allows other species to live in the area
    So beaver and rush act as a facilitator species, presence promotes others presence
    Influences of biotic interactions often involved in Secondary succession

Individualistic vs. interactive hypotheses
Fig. 53.29, (Campbell and Reece, 2005) hypothesis of communities
Individualistic view, that species-species interactions matter little
    what species in an area depend on species sensitivity to abiotic factors?
        such as climate, nutrient conditions, etc...
    this implies the niche of the species is independent of other species;
        Is this R or K strategy?
Interactive hypothesis, that species-species interactions greatly determine community
    In which case other species influence and define a species' niche
        This is more of the K strategy?
Support for both views. Really differs from species to species, and over time

Types of species-species interactions
Community structure often relates to species interactions
    This can define the niche of a species, determining where it lives
So will consider several types of interactions
        Table 54_UN02, interspecific interactions (pg. 1219)
    Competition (-,-)
        for space, nutrients, etc
        competitive exclusion principle may come into play, a type of natural selection?
        this can result in character displacement
            Recall Darwin's finches, specialize to feed on different seeds
                this means they have distinct niches
                fig. 54.4, Finch beak sizes
        Concept of a Niche, use of biotic and abiotic resources, an "ecological space"
        Realized niche vs. fundamental niche
            where does it live in reality, versus where could it potentially live?
            barnacle habitat displacement also an example of competition
                Fig. 54.3, competitive exclusion
        So life in different niches may occur due to competition, and this may alter traits
            see this in a set of lizard species
            Fig. 54.2, lizard partitioning of resources
                see changes in lizard phenotypes associated with distinct niches
            Fig. 54.2, *Anolis distichus*
                lower dwelling
            Fig. 54.2, *Anolis insolitus*
                lives higher up
    Predation (+,-), have same relationship for herbivory and for parasitism...
        Table 54_UN02, interspecific interactions (pg. 1219)
        animal feeding on animal, carnivores
        Plant feeding on animal, and fungi feeding on animal also carnivorous?
        animal on plant, herbivores
            animal feeding on algae also often called herbivory...
        eukaryotes feed on prokaryote, so paramecia are predators...
        parasites, do not kill prey immediately, but still feed on it. specialized predation?
    Mutualism (+,+)
        Example of Acacia trees and Ants
            Fig. 54.7, tree ants and acacia
        Examples of Nitrogen-fixing bacteria and root nodules of plants
            ammonia from bacteria, organic matter from plants
    Commensalism (+,0)
        Table 54_UN02, interspecific interactions (pg. 1219)
        lichen on tree bark?
        barnacles on shells of turtles
        bird feeding in wake of passing herbivores

Coevolution and Invasive Species
     If interactions are strong then two species may show Coevolution
     See this with ants and acacia?
     A new species in an area may avoid interactions,
          or may interact generally with several species.
     Invasive species tend to avoid predators and diseases, so are vigorous and compete well
          Epipactis Helleborine Orchid an example of an invasive species
               (Newcomb, 1977; pg. 49) Epipactis.jpg
               (Chapman 1997; pg. 113) Epipactis helleborine.jpg

Epipactis helleborine **(Orchidaceae)**

By Scott T. Meissner

This orchid (*Epipactis helleborine*) was introduced from Europe. It escaped from gardens and was first reported in the wild in Syracuse, N.Y. in 1879, but it had rapidly spread to California by 1961 (Coleman 1989). It tends to occur in shaded areas, often under pines in less acidic soils. But it has also been reported to grow in urban areas (Champlin 1994).

What traits does this orchid have that made it such a successful invader? For one thing it is a perennial which does not always put up shoots. According to Light et al. (1991) only 7% of the individuals in a population emerge each year. They can do this due to their underground rhizomes that obtain food from fungi. Thus once they arrive in an area they can persist underground until conditions are good. Also, its development is such that its own pollen can deliver sperm to the ovule when the egg is ready to be fertilized (Fredrickson 1992). This ability to inbreed allows those individuals that were dispersed far from other individuals to be able to establish new populations. This independence from cross-pollination means that effective pollinators are not always essential, though Waites et al. (1991) has found that those individuals that have certain secondary floral characteristics can promote pollen exchange between plants and avoid the problems that would come from always inbreeding.

Given its ability to live for a long time underground, a key advantage of this orchid is its ability to associate with some common fungi. But these orchids do not simply have a symbiosis with fungi, they prey on them. This is best seen in strains of this species which are chlorophyll-free and so get all their food from the fungus. According to Salmia (1986, 1988, 1989) these plants have cells with enlarged nuclei in their roots which get very infected with fungi.   These cells digest and consume the fungal hyphae in them, and then recruit more fungi to come in and infect the cells again!   The amazing thing that Salmia reports is that some of these white-colored mutant plants are more vigorous than the normal green strains. So clearly it is able to get lots of material from the fungus. Why would any fungus put up with this abuse? Salmia suggests that the fungus also associates with other plants, especially pines, and that the fungus may get food from the pine which it gives to this orchid to be converted to other needed organic compounds. This promotes the growth of the fungus. The pine would benefit from having the fungus grow more and so being a better supplier to it. A triple-symbiosis.

Meanwhile, at the flowers, this orchid has a different problem. It is mainly pollinated by wasps. They are attracted to the nectar secreted by the flower into its labellum. During these visits they may pick up some pollen, often at the two celled stage in tetrad packets (Vij et al. 1987). But wasps often are good groomers, and so may dislodge most of the pollen on them. However, this orchid also often has microbes growing in its nectar. Many plants secrete antibiotics into their nectar to keep microbes out, but not this orchid. Bacteria and fungi are often brought to the nectar by the wind, or by the visiting wasps. These microbes alter the nectar and create toxins, including ethanol (Ehlers et al. 1997). So after a long relaxing visit and consuming this nectar the wasp staggers out of the flower and fails to groom itself well, and so the flower improves the wasp's efficiency as a pollinator by getting it drunk!

So, how does a plant move to another continent and do well? While being able to inbreed is good, and growing underground for years is nice, it really helps to be good at interacting with other species. Sometimes there are potential interactions which can extend beyond the obvious species involved. In this case there are lectins (proteins with sugars attached to them) from these and other orchids which are being examined closely since they may have anti-HIV activity (Balzarini et al. 1992).

Keystone Species
These are species whose presence or absence have an inordinate affect on a community
> One example is sea otters and killer whales
>> Fig. 54.16, sea otters and killer whales
>> note predation on otters changes urchin and kelp presence
> Book gives example of sea star and mussels, students should look at it...

Species Richness and Species area curves
This is a measure of the number and diversity of species in an area.
Obviously it says something about the structure of the community...
> does it have one dominant species, or several co-dominant species?
How many species can be found in an area? Species/area relationships...
> Area needed to support a number of species
>> Fig. 54.28, species area in islands
> Gives an idea of how much area must be preserved to maintain a certain number of species
>> fig. 54.27, island biogeography
> Note example of large versus small island, and changes in expected numbers of species
>> Effect of distance from mainland on immigration of new species to an island
>> so can balance island size and distance from mainland?
> This implies that small areas differ in
>> species numbers due to competitive exclusion, chance, and other factors
> Consider the implications for conservation biology...
>> how much area would be needed to preserve a certain number of species?

Summary

Communities are real entities...
        Depend in part on
                Frequency and extent of disturbances
                Species-species interactions
                Presence of Top/down influences like keystone species
                Total area involved
                      Etc...

Thus communites represent something larger larger than a species which we would wish to understand...

(Might consider that from the point-of-view of microorganisms, the human body is a wide range of communities, so many of these concepts apply to situations on/in us!)

Objectives:

Be able to define and give examples of various types of interspecific interactions such as commensalism, mutualism, predation, and competition. Be able to describe examples of competitive exclusion and relate this phenomenon to speciation.

Describe the concept of niche, and differentiate between fundamental and realized niche.

How can species interactions help to determine the types of species present in a community? Would the occurance of periodic strong disturbances promote or degrade these interactions? How could such disturbances promote, or inhibit, the presence of a species in an area?

Describe the concept of a keystone species and give an example of it. What is a facilitator species and what does it do?

How do primary and secondary succession differ? Give examples of each.

In what way is the diversity of species in an area dependent on the size of the area and on the amount of disturbance suffered by the area? What other factors might influence the diversity of species found in an area? What uses can be made of the information given in a species/area curve for a community?

Is a biological community a stable entity? What factors promote its stability? What factors make it unstable? What characteristics would you wish to know about if you wished to be able to describe the composition, interactions, and functions of a biological community?

For review, see self-quiz questions #1, 2, 3, 5 and 8 of chapter 54.

Needed overheads and items:

Fig. 54.22, glacial retreat in Alaska
Fig. 54.22, fireweed stage
Fig. 54.22, alder stage
Fig. 54.22, hemlock stage
Table 53.2, (Campbell and Reece, 2002) Succession in Glacier Bay
Fig. 53.21, (Campbell and Reece, 2005) grassland fires
Fig. 54.21. recovery from fire
Fig. 54.17, beavers
Fig. 54.18, black rush influence
Fig. 53.29, (Campbell and Reece, 2005) hypotheses of communities
Table 54.UN02, interspecific interactions (pg. 1219)
Fig. 54.4, Finch beak sizes
Fig. 54.3, competitive exclusion
Fig. 54.2, lizard partitioning of resources
Fig. 54.2, *Anolis distichus*
Fig. 54.2, *Anolis insolitus*
Table 54.UN02, interspecific interactions (pg. 1219)
Fig. 54.7, tree ants and acacia
Table 54.UN02, interspecific interactions (pg. 1219)
(Newcomb, 1977; pg. 49) Epipactis.jpg
(Chapman 1997; pg. 113) Epipactis helleborine.jpg
Fig. 54.16, sea otters and killer whales
Fig. 54.28, species area in islands
Fig. 54.27, island biogeography

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Related issues:

Here are some studies of the effects of **keystone species**, and how their presence or absence can cascade through an ecosystem.

Frank K.T., B. Petrie, J.S. Choi, W.C. Leggett- 2005-Trophic cascades in a formerly cod-dominated ecosystem- Science 308: (6/10) 1621-1623

Morell V- 2007-Aspens return to Yellowstone, with help from some wolves- Science 317: (#5837, 7/27) 438-439

Myers R.A., J.K. Baum, T.D. Shepherd, S.P. Powers, C.H. Peterson- 2007-Cascading effects of the loss of apex predatory sharks from a coastal ocean- Science 315: (3/30) 1846-1850

Naeem S- 2008-Green with complexity- Science 319: (#5865, 2/15) 913-914

Robbins J- 2004-Lessons from the wolf- Scientific American 290: (#6) 76-81

Schmitz O.J- 2008-Effects of predator hunting mode on grassland ecosystem function- Science 319: (#5865, 2/15) 952-954

There are many studies of the effects of various types of **disturbances** on ecosystems and their species. Here are a few examples that include fire, disease, and volcanic erruption.

Bermejo M., J.D. Rodríquez-Teijeiro, G. Illera, A. Barroso, C. Villa, P.D. Walsh- 2006-Ebola out break killed 5000 gorillas- Science 314: (12/8) 1564

Bowman D.M.J.S., J.K. Balch, P. Artaxo, W.J. Bond, J.M. Carlson, M.A. Cochrane, C.M. D'Antonio, R.S. DeFries, J.C. Doyle, S.P. Harrison, F.H. Johnston, J.E. Keeley, M.A. Krawchuk, C.A. Kull, J.B. Marston, M.A. Moritz, I.C. Prentice, C.I. Roos, A.C. Scott, T.W. Swetnam, G.R. van der Werf, S.J. Pyne- 2009-Fire in the Earth System- Science 2009-324: (#5926, 4/24) 481-484

Dale V.H., C.M. Crisafulli, F.J. Swanson- 2005-25 years of ecological change at Mount St. Helens- Science 308: (5/13) 961-962

DelMoral R., I.L. Lacher- 2005-Vegetation patterns 25 years after eruption of Mount St. Helens, Washington, USA- American Journal of Botany 92: (12) 1948-1956

Gaston K.J- 2010-Valuing common species- Science 327: (#5962, 1/8) 154-155

Kerr R.A- 2007-Humongous eruptions linked to dramatic environmental changes- Science 316: (4/27) 527

Vogel G- 2007-Scientists say ebola has pushed western Gorillas to the brink- Science 317: (#5844, 9/14) 1484

Vogel G- 2006-Tracking ebola's deadly march among wild apes- Science 314: (12/8) 1522-1523

Webster P- 2007-Setting the forest alight- Science 317: (#5846, 9/28) 1854-1855

These articles shows how changes in **species-species interactions** can influence **food webs**, and other aspects of an ecosystem.

Barton A.D., S. Dutkiewicz, G. Flierl, J. Bragg, M.J. Follows- 2010-Patterns of diversity in marine phytoplankton- Science 327: (#5972, 3/19) 1509-1511

Burkovinsky T., F.J.F. van Veen, Y. Jongema, M. Dicke- 2008-Direct and indirect effects of resource quality on food web structure- Science 319: (#5864, 2/8) 804-807

deRuiter R.C., V. Wolters, J.C. Moore, K.O. Winemiller- 2005-Food web ecology: Playing jenga and beyond- Science 309; (7/1) 68-70

Hambäck P.A- 2010-A green or a prickly world?- Science 327: (#5973, 3/26) 1583-1584

Lankau R.A., S.Y. Strauss- 2007-Mutual feedbacks maintain both genetic and species diversity in a plant community- Science 317: (#5844, 9/14) 1561-1563

Lerdau M- 2007-A positive feedback with negative consequences- Science 316: (4/13) 212-213

Leslie M- 2008-The importance of being eaten- Science 319: (#5860, 1/11) 146-147

Little C.T.S- 2010-The prolific after life of whales- Scientific American 302: (#2, Feb.) 78-84

Márquez L.M., R.S. Redman, R.J. Rodriquez, M.J. Roossinck- 2007- A virus in a fungus in a plant: Three-way symbiosis required for thermal tolerance- Science 315: (1/26) 513-515

Mooney K.A., R. Halitschke, A. Kessler, A.A. Agrawal- 2010-Evolutionary trade-offs in plants mediate the strength of trophic cascades- Science 327: (#5973, 3/26) 1642-1644

Palmer T.M., M.L. Stanton, T.P. Young, J.R. Goheen, R.M. Pringle, R. Karban- 2008- Breakdown of an ant-plant mutualism follows the loss of large herbivores from an African savana- Science 319: (#5860, 1/11/) 192-195

Persson L., P-A. Amundsen, A.M. DeRoos, A. Klemetsen, R. Kundsen, R. Primiceria- 2007-Culling prey promotes predator recovery-alternative states in a whole-lake experiment- Science 316: (#5833, 6/23) 1743-1746

Sanson G- 2006-The biomechanics of browsing and grazing- American Journal of Botany 93: (#10) 1531-1545

Skelhorn J., H.M. Rowland, M.P. Speed, G.D. Ruxton- 2010-Masquerade: Camouflage without crypsis- Science 327: (#5961, 1/1) 51

Tepe E.J., M.A. Vincent, L.E. Watson- 2007-Stem diversity, cauline domatia, and the evolution of ant-plant associations in *Piper* sect. Macrostachys (Piperaceae)- American Journal of Botany 94: (#1) 1-11

Here is an example of how altering the **interactions** of an insect and a bacterial parasite of it, changes the ability of the insect to carry diseases to humans.

McMeniman C.J., R.V. Lane, B.N. Cass, A.W.C. Fong, M. Sidhu, Y-F. Wang, S.L. O'Neill- 2009-Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*- Science 323: (#5910, 1/2) 141-144

This study looks at whether **species-species interactions** do influence the nature of a **community**.

Kraft N.J.B., R. Valencia, D.D. Ackerly- 2008-Functional traits and niche-based tree community assembly in an Amazonian forest- Science 322: (#5901, 10/24) 580-582

These articles deal with **species-area relationships** both between and within species, and how to evaluate **species richness**.

Clark J.S- 2010-Individuals and the variation needed for high species diversity in forest trees- Science 327: (#5969, 2/26) 1129-1132

DeLaFontaine G., G. Houle- 2007-Species richness along a production gradient: A multivariant approach- American Journal of Botany 94: (#1) 79-88

Ferraz G., J.D. Nichols, J.E. Hines, P.C. Stoufler, R.O. Bierregaard jr., T.E. Lovejoy- 2007-A large-scale deforestation experiment: Effects of patch area and isolation on amazon birds- Science 315: (1/12) 238-241

Here are some nice examples of how one species can influence a **symbiotic** interaction between two other species. So there can be **mutualism** between two species, while one has a **parasitic** affect on yet another species.

Lang A.E., E. Schmidt, A. Schlosser, T.D. Hey, I.M. Larrinua, J.J. Sheets, H.G. Mannherz, K. Aktories- 2010-Photorhabdus luminescens toxins ADP-ribosylate actin and RhoA to force actin clustering- Science 327: (#5969, 2/26) 1139-1142

Oliver K.M., P.H. Degnan, M.S. Hunter, N.A. Moran- 2009-Bacteriophages encode factors required for protection in a symbiotic mutualism- Science 325: (#5943, 8/21) 992-994

Pinto-Tomás A.A., M.A. Anderson, G. Suen, D.M. Stevenson, F.S.T. Chu, W.W. Cleland, P.J. Weimer, C.R. Currie- 2009-Symbiotic nitrogen fixation in the fungus gardens of leaf-cutter ants- Science 326: (#5956, 11/20) 1120-1123

Scott J.J., D-C. Oh, M.C. Yuceer, K.D. Klepzig, J. Clardy, C.R. Currie- 2008-Bacterial protection of beetle-fungus mutualism- Science 322: (#5898, 10/3) 63

Of course some species carry what for them are benign internal species, but which turn out to be pathogens of others (i.e. **zoonosis**). Here is an article on this topic.

Lloyd-Smith J.O., D. George, K.M. Pepin, V.E. Pitzer, J.R.C. Puliam, A.P. Dobson, P.J. Hudson, B.T. Grenfell- 2009-Epidemic dynamics at the human-animal interface- Science 326: (#5958, 12/4) 1362-1367

This article points out how microorganisms may use **antibiotics** for the purpose of **species-species coordination**.

Mlot C- 2009-Antibiotics in nature: Beyond biological warfare- Science 324: (#5935, 6/26)
1637-1639

These articles note how the benefits of avoiding the pressures of **predation** can account for why some animals pay the cost of long distance **migration**.

Gilg O., N.G. Yoccoz- 2010-Explaining bird migration- Science 327: (#5963, 1/15) 276-
277
McKinnon L., P.A. Smith, E. Nol, J.L. Martin, F.I. Doyle, K.F. Abraham, H.G. Gilchrist,
R.I.G. Morrison, J. Bety- 2010-Lower predation risk for migratory birds at high
latitudes- Science 327: (#5963, 1/15) 326-327

Here is a description of how **species diversity** tends to change with **latitude**.

Pennisi E- 2010-On rarity and richness- Science 327: (#5971, 3/12) 1318-1319

BIO 108   2010
Day 14, Lecture 35 Title: Ecosystems: Energy and Mineral Cycling.
Text Readings: Campbell et al. (2008), chapter 55.

Topics to cover:

> **Energy Flow and Productivity**
> **Mineral Cycling**
> > **Studies of Mineral Limits**
> > **Acid Precipitation**
> > **Biomagnification**
> **Nitrogen Cycle**
> **Carbon Cycle**
> **Phosphorous Cycle**
> **Managing productivity**

Flow of energy and minerals through ecosystems. Will consider
        Energy changes, Mineral changes, and how this results in a view of interconnectedness...
The implications of this for agriculture and marine food production will be considered.

Energy Flow and Productivity
Energy Flow through an ecosystem is involved in productivity
        Fig. 54.4, energy flow through hierarchy
                elements can cycle, but energy is a one-way flow, going to heat
Primary producers, bring initial energy into community
        eukaryotic, prokaryotic, anything that does photosynthesis, or chemoautotrophy...
Detritivores liberate chemicals and make them available to the producers
Notice how you could remove all the other stages in this figure and have a functional ecosystem
        with just primary producers and detritivores?
Energy partitioning then follows, energy from producers used to support ecosystem
                Fig. 55.9, energy partitioning
        note that energy lost to heat, waste, etc, with each shift though system
        NPP = GPP - (respiratory and maintenance needs)
Primary productivity of various ecosystems
        varies with location, note rainforests versus polar ice or deserts...
                Fig. 55.6, global net primary productivity
        ocean is not the highest producer on unit area basis, often nutrient starved
                but since is large it contributes alot,
                Some areas in ocean with higher productivity, why?

This figure shows aspects of NPP in a different way
    Fig. 54.4, (Campbell and Reece, 2005) net primary productivity
    Note contribution of algal beds and reefs, why so high?
        high light intensity as are in shallow seas
        high nutrient levels, as lock up nutrients in local minerals and cycle locally
        lots of available water, its an ocean!   ;-)
        stable environment, relative to land this is so...
    Note food grown on cultivated land directly accounts for over 9% of NPP of planet.
Consider rate of turnover in ecosystem, time for a species to alter its population size...
    Forest vs. ocean algal
        which has shorter generation time?
        Which can respond faster to change in env?
        We hear of algal blooms, but seldom of forest blooms?
        Algae come and go in days to weeks, but forests takes decades to centuries
Energy pyramid, as a means to display energy flow through parts of ecosystem
    Production efficiency as move energy from primary to secondary levels...
        net secondary production/assimilation of primary production
        ranges from 1-40%, 10% typical (so called "10%" rule)
        Fig. 55.10, energy pyramid
            loss of energy as move up
            this puts limits on species at upper levels, and on number of trophic levels
            relative stability of lower levels versus instability of higher levels?
Also have pyramids of mass, or numbers...
        Fig. 54.13, (Campbell and Reece, 2005) pyramid of numbers
        Fig. 55.11, pyramid of biomass
    neither of these follows the"10%" rule, but are useful in other ways
    can get an inverted pyramid of mass if turnover rates differ...
        see the caase in the aquatic ecosystem, how fast are the algae turning over?

Mineral Cycling
    Studies of Mineral Limits
Also want to know how matter moves through an ecosystem.
How to study such movements?
Example of Hubbard Brook experiment:
    Fig. 55.16, Hubbard Brook expt.
        follow nutrients in and out, in via rain and mineral decay, out via runoff to streams
            monitored nutrients in stream coming out of a water shed
            several water sheds along the side of this ridge
        Different areas given logging or not
        then can relate this to primary productivity of the area
    So we see that nutrient flow matters

Nutrients can be locked up in biomatter
        so that even in areas of rapid growth nutrients may be limiting
        example of tropical rain forest
        Example, Hubbard Brook, remove trees and more nutrients wash out
        Example, when nutrients are limiting can add them and see changes
                Fig. 55.7, P or N limitation of phytoplankton
                Note how they had to test the samples to determine the limiting factor
Knowing nutrient cycling and flow matters to our economies, are exported with food, etc
        Example, New Zealand exports wool
                Fig. 54.22, (Campbell and Reece, 2002) biomass removal
        wool is protein, so it is high in N.
        Must follow N fixation and fertilizer use carefully
        either limit wool export to rate of native N-fixation in New Zealand
                or import fertilizers to make up the difference.
        Too much N and get eutrophication, too little and yields slowly drop
        Or can try to change the sheep to make more wool on less food...?
      So want to follow nutrient flows as this relates to agricultural productivity
        relates to ecosystem productivity, i.e. agricultural yields, fisheries...

Acid Precipitation

And want to know how our influences on environment often alters nutrient flow
      Example, Acid rain
        Fig. 54.21, (Campbell and Reece, 2005) distribution of acid rain
        Fig. 54.22, (Campbell and Reece, 2005) precipitation and acid rain
        relate to sulfate and nitrate in air pollution, producing acids in rain
             Some of this is put into air via car exhaust
             More put into air from coal-fired power plants, and industrial plants
        Acid replaces cations in soil, displaces calcium ions
             Fig. 5.6 (Taiz and Zeigler, 1991), pH_soil_nutrients.jpg
             For example in area like Hubbard Brook may be depleting calcium ions?
        Changes in soil pH depends on soil type
             in Adirondacks this is causing problems for red spruce...

Biomagnification

Matter moves through ecosystems in different ways. Sometimes can accumulate.
This can result in items at low concentrations at one trophic level of food chain
      being at very high concentrations at another level of a food chain
Example, Biological magnification
            Fig. 55.20, biomagnification
      Also occurs for mercury, PCBs, lead, etc...
      One swordfish effectively consumes items from 100,000 lbs of algae via food chain
        so have to be careful about eating fish now...

Now look at Mineral cycles

    Fig. 55.13, nutrient cycling

    Fig. 54.18, (Campbell and Reece, 2005) generalized biogeochemical cycles

    Note organic and inorganic stages can occur

    Note roles of producers, consumers, and detritivores

    whether a state in the cycle is active or inactive depends on time scale considered?

Nitrogen cycle

    Fig. 55.14, N cycle

    Note changes in redox levels, so once again back to redox reactions...

Compound	Oxidation State of N
NH_3, $-NH_2$	-3
N_2	0
N_2O	+1
NO	+2
NO_2^-	+3
NO_2	+4
NO_3^-	+5

    N-Fixation, N_2 to ammonium (humans do most of this now...)

    Once N is at NH_3 state can be converted into an amine for an amino acid

        Then can act as an electron donor for metabolism

        In our bodies we often use carbon as electron donor

        But many bacteria use nitrogen

        consider Nitrifying bacteria, actually live on energy change available here

    Others use nitrate as an electron acceptor

        denitrifying bacteria

        converts it to N_2, which leaves easily

    The rates of these steps matter

        Consider, a farmer pays for fertilizer, and bacteria release it all to the air!

Carbon Cycle

A major cycle relating to organic compounds

        Fig. 55.14, C cycle

    We have covered C redox states

        recall that metabolism has alot to do with the carbon redox changes

note also has gaseous stage, and that the stages locked up in rocks are not clear here...

Consider Carbon dioxide concentrations in air and green house effects
     Fig. 55.21, CO_2 concentrations and temperatures
Experiments with carbon dioxide availability
     Fig. 55.22, carbon dioxide experiments
     suppliment it and observe local effects on growth rates.
     results are mixed, some species do better for a time (like poison ivy!)
          most show little change in growth rates, as other factors quickly limit their growth

Phosphorus Cycle
This is different from C and N, as it has no gaseous state
          Fig. 55.14, P cycle
     As phosphate ion it is soluble, moves well in streams and to oceans
          but no mobile gaseous form
     so if local minerals lack it can be limiting
     Guano, from birds or bats as phosphate source, also mineral sources
     (Other mineral cycles that lack any gaseous phases are...?)

Managing productivity
So what if want to promote production of a fishing area?
     Fig. 55.6, net primary productivity
Consider first issues relating to mineral flows:
     What does that species of fish eat?
     At what trophic level do these fish occur?
     What do those items need to grow to support the fish?
          Should other species be supported to support these fish?
     What are the production efficiencies?
          Does anything biomagnify?
     What nutrients are limiting at what points?
     Where are those nutrients held or obtained from?
     How to promote their entry into the system without triggering an algal bloom?
Then could consider aspects of the fish population, such as:
     When do they reproduce? When is the best time to harvest the fish?
          What limits to put on such harvests?

Objectives:

Describe the flow of energy and matter through an ecosystem in terms of the producers, consumers, and detritivores. How do the patterns of flow of energy and matter differ, and what processes link the trophic levels? What role do the production efficiencies play in determining the number of individuals that can be supported at higher levels on the energy pyramid? What is biomagnification?

What role does the rate of turnover of primary producers have on the rate of response of an ecosystem to disturbances?

What evidence is there that nutrients are often limiting to the productivity of an ecosystem? Be able to describe specific experimental evidence. Even though the open ocean has plenty of water why does it often show a low net primary productivity when compared to a coastal marsh? What is often present in the marsh that is lacking in the mid-ocean areas?

Be able to describe the nitrogen and carbon cycles. What are the processes of nitrogen fixation, ammonification, nitrification, and denitrification? What process does carbon fixation, and what processes release carbon from organic matter? Where do these processes fit into each cycle?

How is the phosphate cycle different from that of carbon or nitrogen? Why does this have less consequence for ocean ecosystems compared to terrestrial ones?

Be able to describe how the rate of flow of energy and various minerals would be expected to differ in an arctic ecosystem from that in the rainforest, or in various aquatic ecosystems. What biotic and/or abiotic factors account for the differences?

For review, see self-quiz questions #1-3, 5-7 of chapter 55.

Needed overheads and items:

Fig. 55.4, energy flow through hierarchy
Fig. 55.9, energy partitioning
Fig. 55.6, Global net primary productivity
Fig. 54.4, (Campbell and Reece, 2005) net primary productivity
Fig. 55.10, energy pyramid
Fig. 54.13, (Campbell and Reece, 2005) pyramid of numbers
Fig. 55.11, pyramid of biomass
Fig. 55.16b, Hubbard Brook expt.
Fig. 55.16a, Hubbard Brook expt.
Fig. 55.16c, Hubbard Brook expt.
Fig. 55.7, P or N limitation of phytoplankton
Fig. 54.22, (Campbell and Reece, 2002) biomass removal
Fig. 54.21, (Campbell and Reece, 2005) distribution of acid rain
Fig. 54.22, (Campbell and Reece, 2005) precipitation and acid rain
Fig. 5.6 (Taiz and Zeigler, 1991), pH_soil_nutrients.jpg
Fig. 55.20, biomagnification
Fig. 55.13, nutrient cycling
Fig. 54.18, (Campbell and Reece, 2005) generalized biogeochemical cycles
Fig. 55.14, N cycle
Fig. 55.14, C cycle
Fig. 55.21, CO_2 concentrations and temperatures
Fig. 55.22, carbon dioxide experiments
Fig. 55.14, P cycle
Fig. 55.6, net primary productivity

Handout:
Handout - Lecture 35-ecosystems.stm
Fig. 54.4, (Campbell and Reece, 2005) net primary productivity
Fig. 54.13, (Campbell and Reece, 2005) pyramid of numbers

References:

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, R.B.Jackson- 2008-Ecosystems. Chapter 55. Pages 1222-1244. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition. Figures 54.4, 54.13, 54.18, 54.21, 54.22. Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, Figure 54.22. Benjamin Cummings Press. San Francisco, CA.

Taiz L., E. Zeigler- 1991-<u>Plant Physiology</u>. Figure 5.6. Benjamin Cummings Publishing Company, Inc. Redwood City, CA.

Related issues:

The cycling of essential minerals through ecosystems is to our benefit, but the cycling of toxic items through ecosystems can also occur. Mercury (Hg), PCBs, and other items can be **biomagnified**. Here are some studies relating to these issues.

Bergquist B.A., J.D. Blum- 2007-Mass-dependent and -independent fractionation of Hg isotopes by photoreduction in aquatic systems- Science 318: (#5849, 10/19) 417-420

Cristol D.A., R.L. Brasso, A.M. Condo, R.E. Fovargue, S.L. Friedman, K.K. Hallinger, A.P. Monroe, A.E. White- 2008-The movement of aquatic mercury through terrestrial food webs- Science 320: (#5874, 4/18) 335

Kelly B.C., M.G.I. Konomou, J.D. Blair, A.E. Morin, F.A.P.C. Gobas- 2007-Food web-specific biomagnification of persistent organic pollutants- Science 317: (#5835, 7/13) 236-239

Nutrients can also cycle from the **ocean back to the land**. The following articles illustrate this direction of flow.

Blais J.M., L.E. Kimpe, D. McMahon, B.E. Keatley, M.L. Mallory, M.S.V. Douglas, J.P. Smot- 2005-Artic seabirds transport marine-derived contaminants- Science 309: (7/15) 445

Gende S.M., T.P. Quinn- 2006-The fish & the forest- Scientific American 295: (#2, Aug) 84-89

Here are some articles that consider aspects of the **phosphorus** cycle and supplies of it.

Diaz J., E. Ingall, C. Benitez-Nelson, D. Paterson, M.D. deJonge, I. McNulty, J.A. brandes- 2008-Marine polyphosphate: A key player in geologic phosphorus sequestration- Science 320: (#5878, 5/2) 652-655

Vaccari D.A- 2009-Phosphorus: A looming crisis- Scientific American 300; (#6, June) 54-59

For a nice review of the flow of **energy** and **minerals** through ecosystems see:

Molles M.C.jr- 2008-Energy and nutrient relations. Chapter 6, pgs. 133-156. In, Ecology. Concepts & Applications. Fourth edition. McGraw Hill. Boston, MA.

Here are some articles that deal with aspects of the **carbon cycle**.   This is very important for the study of the **greenhouse effect**, and understanding how variable it might be is critical.

Baker D.F- 2007-Reassessing carbon sinks- Science 316: (#5832, 6/22) 1708-1709

Buesseler K.O., C.H. Lamborg, P.W. Boyd, P.J. Lam, T.W. Trull, R.R. Bidigare, J.K.B. Bishop, K.L. Casciotti, F. Dehairs, M. Elskens, M. Honda, D.M. Karl, D.A. Siegel, M.W. Silver, D.K. Steinberg, J. Valdes, B. van Mooy, S. Wilson- 2007- Revisiting carbon flux throught the ocean's twilight zone- Science 316: (4/27) 567-570

Chambers J.Q., J.I. Fisher, H. Zeng, E.L. Chapman, D.B. Baker, G.C. Hurtt- 2007- Hurricane Katrina's carbon footprint on U.S. gulf coast forests- Science 318: (#5853, 11/16) 1107

Dise N.B- 2009-Peatland response to global change- Science 326: (#5954, 11/6) 810-811

Elderfield H- 2010-Seawater chemistry and climate- Science 327: (#5969, 2/26) 1092-1093

Gaillardet J., A. Galy- 2008-Himalaya-carbon sink or source?- Science 320: (#5884, 6/27) 1727-1728

Johnson D., M. Krsek, E.M.H. Wellington, A.W. Stott, L. Cole, R.D. Bardgett, D.J. Read, J.R. Leake- 2005-Soil invertebrates disrupt carbon flow through fungal networks- Science 309: (8/12) 1047

LeQuéré C., C. Rödenbeck, E.T. Buitenhuis, T.J. Conway, R. Langenfelds, A. Gomez, C. Labuschagne, M. Ramonet, T. Nakazawa, N. Metzi, N. Gillet, M. Heimann- 2007- Saturation of the southern ocean CO_2 sink due to recent climate change- Science 316: (#5832, 6/22) 1735-1738

MacDonald G.M., D.W. Beilman, K.V. Kremenetski, Y. Shong, L.C. Smith, A.V. Velichko- 2006-Rapid early development of circumartic peatlands and atmospheric CH_4 and CO_2 variations- Science 314: (10/13) 205-288

Middelburg J.J., F.J.R. Meysman- Burial at sea- Science 316: (6/1) 1294-1295

Rothman D.H., D.C. Forney- 2007-Physical model for the decay and perservation of marine organic carbon- Science 316: (6/1) 1325-1328

Stephens B.B., K.R. Gurney, P.P. Tans, C. Sweeney, W. Peters, L. Bruhwiler, P. Ciais, M. Ramonet, P. Bousquet, T. Nakazawa, S. Aoki, T. Machida, G. Inoue, N. Vinnichenko, J. Lloyd, A. Jordan, M. Heimann, O. Shibistova, R.L. Langenfelds, L.P. Steele, R.J. Francey, A.S. Denning- 2007-Weak northern and strong tropical land carbon uptake from vertical profiles of atmosphere CO_2- Science 316: (#5832, 6/22) 1732-1735

Stone R- 2008-Have desert researchers discovered a hidden loop in the carbon cycle?- Science 320: (#5882, 6/13) 1409-1410

van Oost K., T.A. Quine, C. Govers, S. DeGryze, J.W. Harden, J.C. Ritchie, G.W. McCarty, G. Heckrath, C. Kosmas, J.V. Giraldez, J.R. Margues de Silva, R. Merciex- 2007-The impact of agricultural soil erosion on the global carbon cycle- Science 318: (#5850, 10/26) 626-629

Zhou G., S. Liu, Z. Li, D. Zhang, X. Tang, C. Zhou, J. Yan, J. Mo- 2006-Old-growth forests can accumulate carbon in soils- Science 314: (12/1) 1417

Another major **greenhouse gas** is **methane**, and there is much less known about its sources and sinks. Here are some attempts to start to nail these issues down.

Heimann M- 2010-How stable is the methane cycle?- Science 327: (#5970, 3/5) 1211-1212

Shakhova N., I. Semiletov, A. Salyuk, V. Yusupov, D. Kosmach, O. Gustafsson- 2010- Extensive methane venting to the atmosphere from sediments of the east Siberian arctic shelf- Science 327: (#5970, 3/5) 1246-1250

Nutrients often limit the productivity in many ecosystems, especially in the **open ocean**. This is supported by the **iron** supplimentation experiments. But here is an article that describes the effects of **icebergs** that detach from Antarctic ice fields often supply essential **micronutrients** to Antarctic seas, enhancing the local productivity. Others note the nutrient levels in **current eddies** differ, and alter the local productivity as well. One examines whether the daily cycle of vertical migration of animals in the oceans might bring nutrients to the surface, a process call **biomixing**.

Benitez-Nelson C.R., R.R. Bidigare, T.D. Dickey, M.R. Landry, C.L. Leonard, S.L. Brown, F. Nencioli, Y.M. Rii, K. Maiti, J.W. Becker, T.S. Biddy, W. Black, W-J. Cai, C.A. Carlson, F. Chen, V.S. Kuwahara, C. Mahaffey, P.M. McAndrew, P.D. Quary, M.S. Rappé, K.E. Selph, M.P. Simmons, E.J. Yang- 2007-Mesoscale eddies drive increased silica export in the subtropical pacific ocean- Science 316: (5/18) 1017-1021

Bohannon J- 2007-Sailing the southern sea- Science 315: (3/16) 1520-1521

Boyd P.W., T. Jickells, C.W. Law, S. Blain, E.A. Boyle, K.O. Buesseler, K.H. Coule, J.J. Cullen, H.J.W. deBoar, M. Follows, M. Harvey, C. Lancelot, M. Levasseur, N.P.J. Owens, R. Pollard, R.B. Rivkin, J. Sarmiento, V. Schaemann, V. Smatacek, S. Takeda, A. Tsuda, S. Turner, A.J. Watson- 2007-Mesoscale iron enrichment experiments 1993-2005: Synthesis and future directions- Science 315: (2/2) 612-617

Kerr R.A- 2006-Creatures great and small are stirring the ocean- Science 313: (9/22) 1717

McGillicuddy D.J.jr, L.A. Anderson, N.R. Bates, T. Biddy, K.O. Buesseler, C.A. Carlson, C.S. Davis, C. Ewart, P.G. Falkowski, S.A. Goldthwait, D.A. Hansell, W.J. Jenkins, R. Johnson, V.K. Kosnyrev, J.R. Ledwell, Q.P. Li, D.A. Siegel, D.K. Steinberg- 2007-Eddy/wind interactions stimulated extraordinary mid-ocean plankton blooms- Science 316: (5/18) 1021-1026

Michaels A.F- 2007-Highly active eddies- Science 316: (5/18) 992-993

Smith jr K.L., B.H. Robison, J.J. Helly, R.S. Kaufmann, H.A. Ruhl, J.J. Shaw, B.S. Twing, M. Vernet- 2007-Free-drift icebergs: Hot spots of chemical and biological enrichment in the Weddell Sea- Science 317: (#5837, 7/27) 478-482

Visser A.W- 2007-Biomixing of the oceans?- Science 316: (5/11) 838-839

Here are some articles about the cycling of **sulfur** through ecosystems. It turns out that one form (DMS) made by life in the oceans may influence cloud formation, and perhaps weather.

Howard E.C., J.R. Henriksen, A. Buchan, C.R. Reisch, H. Bürgmann, R. Welsh, W. Ye, J.M. González, K. Mace, S.B. Joye, R.P. Kiene, W.B. Whitman, M.A. Moran- 2006-Bacterial taxa that limit sulfur flux from the ocean- Science 314: (10/27) 649-652

Malia G- 2006-New pieces for the marine sulfur cycle jigsaw- Science 314: (10/27) 607-608

Meskhidze N., A. Nenes- 2006-Phytoplankton and cloudiness in the southern ocean- Science 314: (12/1) 1419-1423

Vallina S.M., R. Simó- 2007-Strong relationship between DMS and the solar radiation dose over the global surface ocean- Science 315: (1/26) 506-508

Vila-Costa M., R. Simó, H. Herada, J.M. Gasol, D. Slezak, R.P. Kiene- 2006- Dimethylsulfoniopropionate uptake by marine phytoplankton- Science 314: (10/27) 652-654

Not all mineral cycling is beneficial to us. Here is a study of how **arsenic** can enter our drinking water.

Fendorf S., H.A. Michael, A. van Geen- 2010-Spatial and temporal variations of groundwater arsenic in South and Southeast Asia- Science 328: (#5982, 5/28) 1123-1127

These are some interesting articles about the **nitrogen cycle** and the uses of fertilizers, sewage treatment, and their affects on ecosystems.

Collins S.L- 2009-Biodiversity under global change- Science 326: (#5958, 12/4) 1353-1354

Duce R.A., J. LaRoche, K. Altieri, K.R. Arrigo, A.R. Baker, D.G. Capone, S. Cornell, F. Dentener, J. Galloway, R.S. Ganesbeam, R.J. Geider, J. Jickells, M.M. Kuypers, R. Langlois, P.s. Liss, S.M. Liu, J.J. Middelburg, C.M. Moore, S. Nickovic, A. Oschlies, T. Pedersen, J. Prospero, R. Schitzer, S. Seitzinger, L.L. Sorensen, M. Uematsu, O. Ulloa, M. Voss, B. Ward, L. Zamora- 2008-Impacts of atmospheric anthropogenic nitrogen on the open ocean- Science 320: (#5878, 5/16) 893-897

Elser J.J., T. Andersen, J.S. Baron, A-K. Bergström, M. Jansson, M. Kyle, K.R. Nydick, L. Steger, D.O. Hessen- 2009-Shifts in lake, N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition- Science 326: (#5954, 11/6) 835-837

Garvin J., R. Buick, A.D. Anbar, G.L. Arnold, A.J. Kaufman- 2009-Isotopic evidence for an aerobic nitrogen cycle in the latest archean- Science 323: (#5917, 2/20) 1045-1048

Galloway J.N., A.R. Townsend, J.W. Erisman, M. Bokunda, Z. Cai, J.R. Freney, L.A. Martinelli, S.P. Seitzinger, M.A Sutton- 2008-Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions- Science 320: (#5878, 5/16) 889-892

Guo J.H., X.J. Liu, Y. Zhang, J.L. Shen, W.X. Han, W.F. Zhang, P. Christie, K.W.T. Goulding, P.M. Vitousek, F.S. Zhang- 2010-Significant acidification in major Chinese croplands- Science 327: (#5968, 2/19) 1008-1010

Kartal B., J.G. Kuenen, M.C.M. van Loosdrecht- 2010-Sewage treatment with anammox- Science 328: (#5979, 5/7) 702-703

McCalley C.K., J.P. Sparks- 2009-Abiotic gas formation drives nitrogen loss from a desert ecosystem- Science 326: (#5954, 11/6) 837-840

Parton W., W.L. Silver, I.C. Burke, L. Grassens, M.E. Harmon, W.S. Currie, J.Y. King, E.C. Adair, L.A. Brandt, S.C. Hart, B. Fasth- 2007-Global-scale similarities in nitrogen release patterns during long-term decomposition- Science 315: (1/19) 361-364

Townsend A.R., R.W. Howarth- 2010-Fixing the global nitrogen problem- Scientific American 302: (#2, Feb.) 64-71

Vance C.P- 2001-Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources- Plant Physiology 127: 390-397

Some have suggested that the higher **carbon dioxide** concentrations in the atmosphere may promote faster plant growth. But this study finds that **nitrogen limitations** may become common and limit plant growth. So nutrient availabilities can interact in complex ways.

Bloom A.J., M. Burger, J.S.R. Asensio, A.B. Cousins- 2010-Carbon dioxide enrichment inhibits nitrate assimilation in wheat and *Arabidopsis*- Science 328: (#5980, 5/14) 899-903

BIO 108   2010
Day 14, Lecture 36, Title: Conservation Ecology.
Text Readings: Campbell et al. (2008), pgs. 1236-1242, and chapter 56.

Topics to cover:

> **Human Views of the World**
> **Habitat Fragmentation**
> **Issues relating to Species Preservation**
> **Extinction and Extinction Rates**
> **Regional/Global Threats**
> **A Personal View...**

Human Views of the World

The biomes of Earth are undergoing great changes, perhaps more changes in a shorter period of
>   time than has occurred in a long time. Most of these changes are driven by human actions,
>   and human actions and views are essential to coping with the problems they create.
How do we view the world? Here is a rather idealized way of looking at it:
>   Fig. 52.19, terrestrial biomes
>   This figure is a false view. More of a historic representation rather than one of reality.
>   Consider:
>   If you have flown across the U.S. you looked down and saw grassland prairie?
>   The tropics may look lush to us, but have been greatly changed.
>   Even the woods here in upstate N.Y. are not at all like what was present centuries
>   ago.
Do we hang on to views of the world as huge and wild as a comfort to ourselves as a way to
>   avoid having to deal with the consequences of our uses of it?
>   Consider: Henry David Thoreau wrote that he was alive (1830s) to see last of wilderness
>   in the northeast US. So what do we now have?
>   Consider: The US Wilderness Act
>   To preserve areas where the "hand of man has never set foot."
>   Is there such a place today?
>   I would argue that there is no wilderness anywhere on the surface of Earth today.

Habitat Fragmentation
Some estimates are that we are using huge resources of the planet.
>   For instance, over a decade ago more than 15% of non-ice covered land space used by us for
>   agriculture (Vitousek et al., 1997)

One result is Habitat Fragmentation, which leaves reduced area for other species to live in...

    Fig. 55.6, (Campbell and Reece, 2002) habitat fragmentation in Wisconsin

    Fig. 55.5, (Campbell and Reece, 2005) fragmentation due to logging

        Increase in edges as well as fragmentation

        Edge of woods may support one species type

            center of woods may support another

    Logging is not the only thing that can produce fragmentation

        Fig. 56.7, habitat fragmentation in suburbia

        Fig. 56.16, corridor over a road

        Roads and suburban developments can separate habitats as well.

    Relate to number of species present per area

    Fig. 53.28, Number of plant species per island area

        A 90% loss of habitat results in 50% loss of species

        with survivors more vulnerble to disturbance

    Can monitor the deforestation by following the reflected (reddish) light from plants via satellite

        Fig. 23_16 Deforestation Molles 2008.jpg

        This example shows how one area of Amazonian forest has been fragmented.

Issues relating to Species Preservation

If we wish to preserve species, what are some issues we must consider?

    Food supplies: Where in the food chain does the species occur?

        Do humans compete with it for that food?

    Genetic Diversity: How large a population is needed to keep it genetically fit over time?

        Ex. Preservation of Illinois prairie chicken population

            Fig. 56.11, prairie chicken population

            Population got so low, was entering an extinction vortex

            Raised genetic diversity, and so population viability,

                by introducing individuals from another population

    Area needed: Obviously elephants need more area than fruit flies

    Range: Does the species migrate? Compete with humans for land useage?

        Fig. 56.18, bear MVP

        Minimum Viable Population, MVP,

            Number estimated to give a certain chance of persistance for

                a given length of time. Typically 95% over 100 years.

            Notice in this figure the area needed for different bear MVPs.

        Constrast this with effective population size (N_e).

    Tolerance of disturbances: What climatic shifts can it tolerate?

        Is there a need for fire, or other disturbances? some species need disturbance

            Fig. 56.13, woodpecker habitat

        Introduced species are often disruptive influences...

            In San Francisco estimate one new species arrives every 12 weeks

                (Vitousek et al., 1997)

        Human dieseases are known to infect apes, complicates ape conservation?

Essential species-species interactions:
     Does it need a specific interaction with some other species?
          Ex: 900 species of fig trees all depend on
               one wasp species as their sole pollinator
     Human interest: Panda bears versus rats?... enough said...
Should efforts be on individual species or on entire ecosystems?
     Instead of focusing on single species try to focus on indicator species.
          Ex. spotted owl, loons
     Need to know which species are keystone species
          Fig. 54.16, keystone species
          need to know species interactions, tolerance for environmental shifts, etc...

Extinction and Extinction Rates
Some estimates state that humans interact with directly or indirectly 60-90% of the primary
     productivity of the planet by using food, plant material in field, harvested fish, managed
     woods etc... This leaves little for other species not being used by us...
Review estimates of extinction rates. (Gibbs, 2001)
     1.8 million species known. May be up to 10X more to be identified!
     Many extinctions therefore may go unrecorded.
     E.O. Wilson suggested an extinction rate of 1 species per 30 minutes
          2/hour, 48/day, or about 10000/year
     E.O. Wilson (1992) by 2020, may lead to 20% of species becoming extinct?
     Some say this rate of extinction is 1000X > than that before humans existed
          (Vitousek et al., 1997).
     Others dispute this, but most agree it is high and rising.
A few specific examples of extinctions driven by direct human actions (Balouet and Alibert, 1990)
     This source identifies over 600 known species of vertebrates driven to extinction
     South Indian Ocean Islands: no humans, Reunion, Mauritius, Rodriguez Island
          discovered by European explorers in 1500s
        pg 41, (Balouet etal, 1990) Indianocean.jpg
        Ex: elephant bird
          pg. 51 (Balouet etal, 1990) elephandbird.jpg
          Egg was 13" wide, some kept in collections...
          Hunted to extinction before 1800s
        Ex: Giant tortoise
          pg. 53 (Balouet etal, 1990) tortoise.jpg
          taken for food on the hoof
          could live 15 weeks in ship's hold with no
               water or food and then eaten
          "... so numerous thoughout the island that one person could kill 1200 a day, or
               to put it more strongly, as many as he liked."
               - 1671, de Lespinay -

Ex: Dodo

pg. 43 (Balouet etal, 1990) dodo.jpg

also hunted for food

"The longer they were cooked, the more insipid and tough their flesh became." Van Neck, 1596.

Could go into other species greatly reduced such as whales, seals, buffalo, etc.

Regional/Global Threats

Human actions now working on a much broader level than individual species.

Threats exist to entire ecosystems...

These include:

Acid rain, forest dieback

fig. 54.21, (Campbell and Reece, 2005) acid rain

alters soil chemistry, and so viability of plants in these areas...

this has wiped out much of the red spruce in the Adirondack forests....

Global warming, earlier spring in the North east U.S. (Miller-Rushing et al., 2006)

Miller-Rushing Global Warming Fig 1.jpg

Just a small change in average temperature in spring in Massachuetts area

Miller-Rushing Global Warming Fig 2.jpg

Resulted, so far, in leafing out and flowering almost one month earlier!

Review that causes this, greenhouse gases and flow of energy

Fig 23_2 Greenhouse Effect Molles 2008.jpg

Greenhouse gases include: Methane, CO_2, N_2O, water vapor, etc...

Note effects on different wavelengths of light

What does warming of oceans do to its ability to hold dissolved oxygen?

What will this do to fish?

What does carbon dioxide do when it is in water?

What happens to limestone ($CaCO_3$) when exposed to such water?

What are coral reefs made out of?

One estimate is that in 50 years there may be no coral reefs like today!

UV and ozone depletion

Ozone protects us from ultraviolet radiation

Fig. 55.25, ozone hole

If lose our UV barrier will lose many land species.

CFC (clorofluorocarbon) production mostly banned,

Fig. 55.23, ozone layer

but not in India and China and many other countries

a case of economic needs overruling environmental safety?

Water Pollution, see large dead zones in the oceans (Mee, 2006)

Eutrophication, excess N and P, is one cause.

Fig. 55.18, dead zones

Normal shallow sea has dissolved oxygen gas to bottom

Mee 2006 pg 82.jpg

With mineral introduction the surface water productivity rises,

makes organic matter that drops to the bottom and is respired

produces anoxic conditions in bottom waters

Mee 2006 pg 83.jpg

see shift in food web, more jellyfish, less commerically useful fish

Black sea has had several dead zones, with over 40000 Km^2 area altered

Mee 2006 pg 78.jpg

See many other dead zones inother regions of the oceans

Mee 2006 pg 80.jpg

Oceans also losing coral reefs, also threatened by trash such as plastics... etc...

A Personal View...

Fig. 56.2, tropical deforestation

I believe that there is no wilderness anymore on earth.

  There are only areas we choose to use alot, or to use a little.

  A park, a preserve, a zoo... These are good attempts at preservation,

   but are not wilderness

  There are no areas in the biome of Earth that humans have not influenced

Some argue that we should cut back our consumption, and even our population,

  to allow more of the world's productivity to be left for other species and ecosystems.

  Certainly we may not need 6.8 billion people to have a viable human civilization?

   Fig. 53.22, human population growth

  Certainly most of these environmental problems would be easier to manage if

   the total human population was just 100 million?

   But the population is currently still growing, not shrinking at all...

    Fig. 1, World Population projections (UN World population, 2005)

   If all human couples limited themselves each to one child,

    by 2100 the world population would be about 1.5 billion.

  What is the likelyhood of humans making these changes....?

Therefore, species have been driven extinct, and will be driven extinct,

  and the wonder of the world will be reduced... and it will be due to our actions.

Any species has the right to fill their niche, we are filling ours.

   Ours is just the whole world... it seems...

But we claim to be more than just a species of bacteria filling the petri dish of the world...

  We claim to the apex of evolution, we claim moral and intellectual superiority, and many

   of us claim divine right to make the world conform to our vision.

  The species we drive to extinction are our relatives, our cousin species.

   They are also a source of wonder to us. They make our world beautiful.

Therefore, some argue that we have an obligation to build a human civilzation that is worthy of

  the sacrifices we impose on our relatives.

  Humans must build great cities, compose beautiful music, write great books,

   we must seek to balance the wonder we destroy with wonder we create...

  There are things we can do that no other species can attempt

   Example: Send species to other solar systems (Alexander 2009).

    We should do this...   and other things.

The value of your work depends to some extent on what your work is in service of.

I suggest that the life of this planet is rare and wonderful, and fully deserving of your service.

Biologists, to one degree or another, all share a feeling of awe for the diversity and wonder of life.

Many offer their work up to understand, manage, and attempt to preserve or honor that life.

If you become a doctor, engineer, or other professional, you will be, as a professional, doing it as a form of service. Your service to our civilization is important, but your service need not be only for humans, but in service of life, of which humans are a part as we craft a civilization and attempt to do things that enhance the wonder and beauty of the world.

"Apply your mind to at least one problem which has never been solved, which in general is considered impossible of solution, but which, being solved, would help humanity. Do with your life something that has never been done, but which you feel needs doing."

   -Harvey H. Nininger-

"If I told you that I was going to magically introduce a gene into the human population that would make all your grandchildren and all their descendants color blind, you would be less than pleased. The same is true of a gene that would eliminate all awareness of music. But what are color vision and music awareness? Traits that you would never know you had if the world was not colored and the air not filled with complex sonorous sounds. I maintain that you, dear reader, are an animal rich in mental and physical receptors for the complexity of nature; and by destroying that nature, you condemn your offspring to the sleep of never even knowing those receptors exist -- and by destroying tropical nature, you destroy easily the majority of the signals those receptors are designed to receive. Of course, humans are good at generating mild complexity in their workings. But the level of complexity generated by humanity is to the complexity of a tropical forest as a mouse's squeak is to all of human music. The city-center dweller who feels no cultural and biological deprivation is simply unaware of what he is missing. If your response is, well, what he doesn't know won't hurt him, then I suppose that you won't mind if I eliminate color vision from your children at birth, and all their children after them. Humans have spent rather many millions of years inventing the ability to be very aware of what is around them. How ironic that just about the time they get themselves to where they can sit back and gawk rather than fear all of that, their quintessential human trait is removing the very thing that made them what they are. A human without sense is not even an animal."

   - Professor Daniel H. Janzen, University of
      Pennsylvania, in a 1982 paper to the Office of
      Technology Assessment -

Objectives:

Habitat fragmentation causes the loss of habitat and an increase in "edges" or transition regions between habitats. What influence does this have on efforts to conserve species? Be able to relate species/area relationships to attempts at conservation.

What knowledge of the ecological characteristics of a species need to be known in order to preserve it as well as the ecosystem in which it lives? Understand the concepts of MVP and N_e, and be able to relate them to efforts to maintain a small population. Do any species depend upon disturbance in order to survive? Would such disturbance be left to occur by chance, or must humans often supply it? What are keystone species, and what role might they play in conservation efforts?

Be able to describe the greenhouse effect and how it drives global warming. What gases are greenhouse gases? Be able to describe other specific regional/global environmental changes that threaten the survival of either individual species or entire ecosystems.

Gain a sense of the rate of species extinction, and various causes of extinction.

Decide what you feel you should do in the face of such challenges to the quality of human existence.

For review, see self-quiz questions #1, 4, 6, 7 and 8 of chapter 56.

Needed overheads and items:

Fig. 52.19, terrestrial biomes
Fig. 55.6, (Campbell and Reece, 2002)   habitat fragmentation in Wisconsin
Fig. 55.5, (Campbell and Reece, 2005) fragmentation
Fig. 56.7, suburban habitat fragmentation
Fig. 56.16, corridor between two divided habitats
Fig. 53.28, (Campbell and Reece, 2005) Number of plant species per island area
Fig. 23_16 Deforestation Molles 2008.jpg
Fig. 56.11, prairie chicken conservation
Fig. 56.18, bear MVP
Fig. 56.13, woodpecker habitat
Fig. 54.16, keystone species
pg 41, (Balouet etal, 1990) Indianocean.jpg
pg. 51 (Balouet etal, 1990) elephandbird.jpg
pg. 53 (Balouet etal, 1990) tortoise.jpg
pg. 43 (Balouet etal, 1990) dodo.jpg
fig. 54.21, (Campbell and Reece, 2005) acid rain
Miller-Rushing Global Warming Fig 1.jpg
Miller-Rushing Global Warming Fig 2.jpg
Fig 23_2 Greenhouse Effect Molles 2008.jpg
Fig. 55.25, ozone hole
Fig. 55.23, ozone layer
Fig. 55.18, ocean dead zone
Mee 2006 pg 82.jpg
Mee 2006 pg 83.jpg
Mee 2006 pg 78.jpg
Mee 2006 pg 80.jpg
Fig. 56.2, tropical deforestation
Fig. 53.22, human population growth
Fig. 1, World Population projections (UN World population, 2005)

References:

Alexander A- 2009-Who will survive? Ten hardy organisms selected for the LIFE project-
    The Planetary Report 29: (#2, Mar/Apr) 4-9

Balouet J-C., E. Alibert- 1990-<u>Extinct Species of the World</u>. (English translation) Barron's
    Educational Series, Inc. Hauppauge, N.Y.

Campbell N.A., J.B.Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky,
    R.B.Jackson- 2008-Conservation biology and restoration ecology. Chapter
    56, and pgs. 1236-1242. <u>Biology</u>. Eighth edition. Pearson/Benjamin Cummings
    Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2005-<u>Biology</u>, 7th edition. Figures 53.28, 54.21, 55.5.
    Benjamin Cummings Press. San Francisco, CA.

Campbell N.A., J.B. Reece- 2002-<u>Biology</u>, 6th edition, figure 55.6. Benjamin Cummings
    Press. San Francisco, CA.

Gibbs W.W- 2001-On the termination of species- Scientific American 285: (#5) 40-49

Mee L- 2006-Reviving dead zones- Scientific American 295: (#5, Nov) 78-85

Miller-Rushing A.J., R.B. Primack, D. Primack, S. Mukunda- 2006-Photographs and
    herbarium specimens as tools to document phenological changes in response to
    global warming- American Journal of Botany 93: (#11) 1667-1674

Molles M.C.jr- 2008-<u>Ecology. Concepts & applications</u>. Fourth edition. Figs. 23.2,
    23.16. McGraw Hill. Boston, MA.

Wilson E.O- 1992-<u>The Diversity of Life</u>. 424 pgs. Belknap Press. Cambridge, MA.

United Nations Secretate- 2005-World Population Prospects: The 2004 Revision.
    Department of Economic and Social Affairs. N.Y., N.Y.
    (www.unpopulation.org)

Vitousek P.M., H.A. Mooney, J. Labchenco, J.M. Melillo-1997-Human domination of
    Earth's ecosystems- Science 277: (#5325, 7/25) 494-499

Related issues:

Various ways to deal with environmental problems:

One way to deal with **sea level rise** might be to hold more water in reservoirs.

Chao B.F., Y.H. Wu, Y.S. Li- 2008-Impact of artificial reservoir water impoundment on global sea level- Science 320: (#5873, 4/11) 212-214

New **energy sources** will be needed. One suggestion concerns nuclear fusion by a new approach. Or, if you could recharge batteries while you walk, would you? Biofuels have been pushed recently, while the results are not all that was desired new approaches are possible.

Donelan J.M., Q. Li, V. Naing, J.A. Hoffer, D.J. Weber, A.D. Kuo- 2008-Biomechanical energy harvesting: Generating electricity during walking with minimal user effort- Science 319: (#5864, 2/8) 807-810

Fargione J., J. Hill, D. Tilman, S. Polasky, P. Hawthorne- 2008-Land clearing and the biofuel carbon debt- Science 319: (#5867, 2/29) 1235-1238

Hirsch R.L- 1967-Inertial-electrostatic confinement of ionized fusion gases- Journal of Applied Physics 38: (#11, Oct.) 4522-4534

Hurst J.K- 2010-In pursuit of water oxidation catalysts for solar fuel production- Science 328: (#5976, 4/16) 315-316

Ligon T- 2008-The world's simplest fusion reactor revisited- Analog: Science Fiction and Fact 128: (#1/2, Jan/Feb) 60-75

Righelato R., D.V. Spracklen- 2007-Carbon mitigation by biofuels or by saving and restoring forests?- Science 317: (#5840, 8/17) 902

Scharlemann J.P.W., W.F. Laurance- 2008-How green are biofuels?- Science 319: (#5859, 1/4) 43-44

Searchinger T., R. Heimlich, R.A. Houghton, F. Dong, A. Elobeid, J. Fobiosa, S. Tokgaz, D. Hayes, T-H. Yu- 2008-Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change- Science 319: (#5867, 2/29) 1238-1240

Service R.F- 2007-Biofuel researchers propose to reap a new harvest- Science 315: (3/16) 1488-1491

Stone R- 2007-Can palm oil plantations come clean?- Science 317: (#5844, 9/14) 1491

Tilman D., J. Hill, C. Lehman- 2006-Carbon-negative biofuels from low-input high-diversity grassland biomass- Science 314: (12/8) 1598-1600

Big problems need big solutions. **Global environmental engineering** approaches are being proposed.

Angel R- 2006-Feasibility of cooling the Earth with a cloud of small spacecraft near the inner Lagrange point (L1)- Proceedings of the National Academy of Science (USA) 103: (#46, Nov. 14) 17184-17189

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Kintisch E- 2007-Tinkering with the climate to get hearing at Harvard meeting- Science 318; (#5850, 10/26) 551

Kintisch E- 2007-Scientists say continued warming warrants closer look at drastic fixes- Science 318: (#5853, 11/16) 1054-1055

Kunzig R- 2008-A sunshade for planet Earth- Scientific American 299: (#5, Nov) 46-55

Tilmes S., R. Müller, R. Salawitch- 2008-The sensitivity of polar ozone depletion to proposed geoengineering schemes- Science 320: (#5880, 5/30) 1200-1204

Wigley T.M.L- 2006-A combined mitigation/geoengineering approach to climate stabilization- Science 314: (10/20) 452-454

Efforts are made to **perserve ecosystems**, and the species in them or how to restore or manage them. Here are a few examples.

Barbier E.B., E.W. Koch, B.K. Silliman, S.D. Hacker, E. Wolanski, J. Primavera, E.F. Granek, S. Polasky, S. Aswani, L.A. Cramer, D.M. Stoms, C.J. Kennedy, D. Bael, C.V. Kappel, G.M.E. Perillo, D.J. Reed- 2008-Coastal ecosystem-based management with nonlinear ecological functions and values- Science 319: (#5861, 1/18) 321-323

Butler S.J., J.A. Vickery, K. Norris- 2007-Farmland biodiversity and the footprint of agriculture- Science 315: (1/19) 381-384

Donald P.F., F.J. Sanderson, I.J. Burfield, S.M. Bierman, R.D. Gregory, Z. Waliczky- 2007-International conservation policy delivers benefits for birds in Europe- Science 317: (#5839, 8/10) 810-813

Doyle M.W., E.H. Stanley, D.G. Havlick, M.J. Kaiser, G. Steinbach, W.L. Graf, G.E. Galloway, J.A. Riggshee- 2008-Aging infrastructure and ecosystem restoration- Science 319: (#5861, 1/18) 286-287

Hilbom R., R. Arsese, M. Bomer, J. Hendo, G. Hopcraft, M. Loibooki, S. Mduma, A.R.E. Sinclair- 2006-Effective enforcement in a conservation area- Science 314: (11/24) 1266

Kareiva P., S. Watts, R. McDonald, T. Boucher- 2007-Domesticated nature: Shaping landscapes and ecosystems for human welfare- Science 316: (#5833, 6/29) 1866-1869

Kareiva P., M. Marvier- 2007-Conservation for the people- Scientific American 297: (#4, Oct.) 50-57

Kreman C., A. Cameron, A. Moilanen, S.J. Phillips, C.D. Thomas, H. Beentje, J. Dransfield, B.L. Fisher, F. Glaw, T.C. Good, G.J. Harper, R.J. Hijmans, B.C.

Lees, E. Lewis jr., R.A. Nussbaum, C.J. Raxworthy, A. Razafimpahanana, G.E. Schatz, M. Vences, D.R. Vieites, P.C. Wright, M.L. Zjhra- 2008-Aligning conservation priorities across taxa in Madagascar with high-resolution planning tools- Science 320: (#5873. 4/11) 222-226

Morell V- 2008-Wolves at the door of a more dangerous world- Science 319: (#5865, 2/15) 890-892

Myers M.D., M.A. Ayers, J.S. Baron, P.R. Beauchemin, K.T. Gallagher, M.B. Goldhaber, D.R. Hutchinson, J.W. LaBaugh, R.G. Sayre, S.E. Schwarzbach, E.S. Schweig, J. Thormodsgard, C. van Riper III, W. Wilde- 2007-USGS goals for the coming decade- Science 318: (#5848, 10/12) 200-201

Nepstad D., B.S. Soares-Filho, F. Merry, A. Lime, P. Moutinho, J. Carter, M. Bowman, A. Cattaneo, H. Rodrigues, S. Schwartzman, D.G. McGrath, C.M. Stickler, R. Lubowski, P. Piris-Cabezas, S. Rivero, A. Alencar, O. Almeida, O. Stella- 2009-The end of deforestation in the Brazilian Amazon- Science 326: (#5958, 12/4) 1350-1351

Oliveira P.J.C., G.P. Asner, D.E. Knapp, A. Almeyda, R. Galván-Gildemeistar, S. Keene, R.F. Raybin, R.C. Smith- 2007-Land-use allocation protects the Peruvian amazon- Science 317: (#5842, 8/31) 1233-1236

Phelps J., E.L. Webb, A. Agrawal- 2010-Does REDD threaten to recentralize forest governance?- Science 328: (#5976, 4/16) 312-313

Rodríguez J.P., A.B. Taber, P. Daszak, B. Sukumar, C. Valladares-Padua, S. Padua, L.F. Aguirré, R.A. Medellin, M. Acosta, A.A. Aquirre, C. Bonacic, P. Bordino, J. Bruschini, D. Buchori, S. González, T. Mathew, M. Méndez, L. Mugica, L.F. Pacheco, A.P. Dobson, M. Pearl- 2007-Globalization of conservation: A view from the south- Science 317: (#5839, 8/10) 755-756

Stokstad E- 2010-Science meets politics off California's coast- Science 327: (#5973, 3/26) 1574-1575

Stone R- 2008-Show down over a biological treasure trove- Science 319: (#5870, 3/21) 1604

Wang G., J.L. Innes, J. Lei, S. Dai, S.W. Wu- 2007-China's forestry reform- Science 318: (#5856, 12/7) 1556-1557

Wuethrich B- 2007-Reconstructing Brazil's atlantic rainforest- Science 315: (2/23) 1070-1072

Attempts at conservation need to be evaluated to determine what is working and what is not. This study finds that an **international treaty** failed to meet the goals it set that were meant to conserve biodiversity.

Butchart S.H.M., M. Walpole, B. Collen, A. van Strien, J.P.W. Scharlemann, R.E.A. Almond, J.E.M. Baillie, B. Bomhard, C. Brown, J. Bruno, K.E. Carpenter, G.M. Carr, J. Chanson, A.M. Chenery, J. Csirke, N.C. Davidson, F. Dentener, M. Foster, A. Galli, J.N. Galloway, P. Genovesi, R.D. Gregory, M. Hockings, V. Kapos, J-F. Lamarque, F. Leverington, J. Loh, M.A. McGeoch, L. McRae, A. Minasyan, M.H. Morcillo, T.E.E. Oldfield, D. Pauly, S. Quader, C. Revenga, J.R. Sauer, B. Skolnik, D. Spear, D. Stanwell-Smith, S.N. Stuart, A. Symes, M. Tierney, T.D. Tyrrell, J-C. Vié, R. Watson- 2010-Global biodiversity: Indicators of recent declines- Science 328: (#5982, 5/28) 1164-1168

This article describes the use of nuclear transfer and other genetic technologies to try to **preserve the genetics** of species that are going extinct, in this case salmon.

Okutsu T., S. Shikina, M. Kanno, Y. Takeuchi, G. Yoshizaki- 2007-Production of trout offspring from triploid salmon parents- Science 317: (#5844, 9/14) 1517

Ways to reduce greenhouse gas emissions are being sought. The "ivory tower" offers up virtual meetings to avoid the **carbon emissions** of travel to meetings.

Lester B- 2007-Greening the meeting- Science 318: (#5847, 10/5) 36-38

Using the power of **capitalism**, one approach is to move towards including the costs to the environment into national GDPs.

Liu J., J. Diamond- 2008-Revolutionizing China's environmental protection- Science 319: (#5859, 1/4) 37-38

This article notes that with various changes in the region, the rate of **tree mortality** of the forests in the western part of North American has risen.

van Mantgem P.J., N.L. Stephensen, J.C. Byrne, L.D. Daniels, J.F. Franklin, P.Z. Fulé, M.E. Harmon, A.J. Larson, J.M. Smith, A.H. Taylor, T.T. Veblen- 2009-Widespread increase of tree mortality rates in the Western United States- Science 323: (#5913, 1/23) 521-524

Most of our water use is for agriculture, as **freshwater** becomes limiting there are more moves being made toward the use of **desalinized sea water**.

Rogers P- 2008-Facing the freshwater crisis- Scientific American 299: (#2, Aug) 46-53

Yermiyahu U., A. Tal, A. Ben-Gal, A. Bar-Tal, J. Tarchitzky, O. Lahav- 2007-
Rethinking desalinated water quality and agriculture- Science 318: (#5852, 11/9) 920-921

Some suggest that to respond to changes in the environment a shift from annual crops to perennials might reduce the environmental impacts on **agriculture**. Other suggestions involve changes in irrigation practices.

Gleick P.H- 2001-Making every drop count- Scientific American 284: (#2, Feb) 40-45

Glover J.D., C.M. Cox, J.P. Reganold- 2007-Future farming: A return to roots?-
Scientific American 297: (#2, Aug.) 82-89

Godfray H.C.J., J.R. Beddington, I.R. Crute, L. Haddad, D. Lawrence, J.F. Muir, J. Pretty, S. Robinson, S.M. Thomas, C. Toulmin- 2010-Food security: The challenge of feeding 9 billion people- Science 327: (#5967, 2/12) 812-818

Postel S- 2001-Growing more food with less water- Scientific American 284: (#2, Feb) 46-49

Wullschleger S.D., M. Strahl- 2010-Climate change: A controlled experiment- Scientific American 302: (#3, March) 78-83

One of the approaches to address local pollution is the use of microbes or other organisms that are bio-engineered to take up the pollutant, pulling it out of the soil or dispersing it. This is called **bioremediation**. Here is an example.

Omichinski J.G- 2007-Toward methyl mercury bioremediation- Science 317: (#5835, 7/13) 205-206

There are attempts to **catalog** the diversity of life on earth. Here is one example.

Stuart S.N., E.O. Wilson, J.A. McNeely, R.A. Mittermeier, J.P. Rodriguez- 2010-The barometer of life- Science 328: (#5975, 4/9) 177

And now, some of the challenges we face:

Global climate change is now well documented. Here are some recent reports of its past and others attempting to anticipate future effects.

Barnett T.P., D.W. Piéreo, K.M. AchutaRao, P.J. Glocker, B.D. Santer, J.M. Gregory, W.M. Washington- 2005-Penetration of human-induced warming into the world's oceans- Science 309: (7/8) 284-287

Battisti D.S., R.L. Naylor- 2009-Historical warnings of future food insecurity with unprecedented seasonal heat- Science 323: (#5911, 1/9) 240-244

Bierbaum R.M., P.H. Raven- 2007-A two-pronged climate strategy- Science 315: (4/6) 17

Bohannon J- 2008-The big thaw reaches Mongolia's pristine north- Science 319: (#5863, 2/1) 567-568

Codispoti L.A- 2010-Interesting times for marine N_2O- Science 327: (#5971, 3/12)

Collins W., R. Colman, J. Haywood, M.R. Manning, P. Mote- 2007-The physical science behind climate change- Science 297: (#9, Aug.) 64-73

Cox P., C. Jones- 2008-Illuminating the modern dance of climate and CO_2- Science 321: (#5896, 9/19) 1642-1644

Fox D- 2007-Back to the no-analog future?- Science 316: (5/11) 823-825

Grossman D- 2004-Spring forward- Scientific American 290: (#1) 84-91

Hansen J., L. Nazarenko, R. Ruedy, M. Sato, J. Willis, A. Del Genio, D. Koch, A. Lacis, K. Lo, S. Menon, T. Novakov, J. Perlwitz, G. Russell, G.A. Schmidt, N. Tausner- 2005-Earth's energy imbalance: Confirmation and implications- Science 308: (6/3) 1431-1435

Hansen J- 2004-Defusing the global warming time bomb- Scientific American 290: (#3) 68-77

Keeling R.F- 2008-Recording Earth's vital signs- Science 319: (#5871, 3/28) 1771-1772

Keppler R., T. Röckmann- 2007-Methane, plants and climate change- Scientific American 296: (#2, Feb) 52-57

Kerr R.A- 2007-Global warming comes home to roost in the American west- Science 318: (#5858, 12/21) 1859

Kerr R.A- 2007-How urgent is climate change?- Science 318: (#5854, 11/23) 1230-1231

Kerr R.A- 2007-Is battered arctic sea ice down for the count?- Science 318: (#5847, 10/5) 33-34

Kerr R.A- 2007-Record U.S. warmth of 2006 was part natural, part greenhouse- Science 317: (#5835, 7/13) 182-183

Kerr R.A- 2007-Pushing the scary side of global warming- Science 316: (6/8) 1412-1415

Kerr R.A- 2007-Global warming is changing the world- Science 316: (4/13) 188-190

Kintisch E- 2008-Roads, ports, rails aren't ready for changing climate, says report- Science 319: (#5871, 3/28) 1744-1745

Kömer C., D. Basler- 2010-Phenology under global warming- Science 327: (#5972, 3/19) 1461-1462

Malhi Y., J.T. Roberts, R.A. Betts, T.J. Killeen, W. Li, C.A. Nobre- 2008-Climate change, deforestation, and the fate of the amazon- Science 319: (#5860, 1/11) 169-172

Milly P.C.D., J. Betancourt, M. Falkenmark, R.M. Hirsch, Z.W. Kundzewicz, D.P. Lettenmaier, R.J. Stouffer- 2008-Stationary is dead: Whither water management?- Science 319: (#5863, 2/1) 573-574

Moritz C., J.L. Patton, C.J. Conroy, J.L. Parra, G.C. White, S.R. Beissinger- 2008-Impact of a century of climate change on small-mammal communities in Yosemite National Park, USA- Science 322: (#5899, 10/10) 261-264

Oerlemans J- 2005-Extracting a climate signal from 169 glacier records- Science 308: (4/29) 675-677

Oppenheimer M., B.C. O'Neill, M. Webster, S. Agrawala- 2007- The limits of concensus- Science 317: (#5844, 9/14) 1505-1506

Patt A.G., L. Ogallo, M. Hellmuth- 2007-Learning from 10 years of climate outlook forums in Africa- Science 318: (#5847, 10/5) 49-50

Pennesi E- 2005-Beloved artic station braces for its own climate change- Science 309: (8/12) 1006-1008

Pörtner H.O, A.P. Farrell- 2008-Physiology and climate change- Science 322: (#5902, 10/31) 690-692

Primack D., C. Imbres, R.B. Primack, A.J. Miller-Russing, P.D. Tredici- 2004- Herbarium specimens demonstrate earlier flower times in response to warming in Boston- American Journal of Botany 91: (#8) 1260-1264

Raes R., R. Swart- 2007-Climate assessment: What's next?- Science 318: (#5855, 11/30) 1386

Rahmstorf S., A. Cazenave, J.A. Church, J.E. Hansen, R.F. Keeling, D.E. Parker, R.C.J. somerville- 2007-Recent climate observations compared to projections- Science 316: (5/4) 709

Roe G.H., M.B. Baker- 2007-Why is climate sensitivity so unpredictable?- Science 318: (#5850, 10/26) 629-632

Seager R., M. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H-P. Huang, N. Harnik, A. Leetmaa, N-C. Lau, C. Li, J. Velez, N. Naik- 2007-Model projetions of an imminent transition to a more arid climate in south western North America- Science 316: (5/25) 1181-1184

Serreze M.C., M.M. Holland, J. Stroeve- 2007-Perspectives on the Arctic's shrinking sea-ice cover- Science 315: (3/16) 1533-1536

Shine K.P., W.T. Sturges - 2007-CO_2 is not the only gas- Science 315: (3/30) 1804-1805

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rate of global warming- Science 327: (#5970, 3/5) 1219-1223

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Wang T., J. Overgaard- 2007-The heartbreak of adapting to global warming- Science 315: (1/5) 49-50

Wentz F.J., L. Ricciardulli, K. Hilburn, C. Mears- 2007-How much more rain will global warming bring?- Science 317: (#5835, 7/13) 233-235

Zeng N., Y. Ding, J. Pan, H. Wang, J. Gregg- 2008-Climate change - The Chinese challenge- Science 319: (#5864, 2/8) 730-731

There are many who are **skeptical about global warming**. Here is one of the best responses to those skeptics that I have encountered written by "a few" researchers.

Gleick P.H., R.M. Adams, R.M. Amasino, E. Anders, D.J. Anderson, W.W. Anderson, L.E. Anselin, M.K. Arroyo, B. Asfaw, F.J. Ayala, A. Bax, A.J. Bebbington, G. Bell, M.V.L. Bennett, J.L. Bennetzen, M.R. Berenbaum, O.B. Berlin, P.J. Bjorkman, E. Blackburn, J.E. Blamont, M.R. Botchan, J.S. Boyer, E.A. Boyle, D. Branton, S.P. Briggs, W.R. Briggs, W.J. Brill, R.J. Britten, W.S. Broecker, J.H. Brown, P.O. Brown, A.T. Brunger, J. Cairns jr., D E. Canfield, S R. Carpenter, J.C. Carrington, A R. Cashmore, J.C. Castilla, A. Cazenave, F.S. Chapin III, A.J. Ciechanover, D.E. Clapham, W.C. Clark, R.N. Clayton, M.D. Coe, E.M. Conwell, E.B. Cowling, R.M Cowling, C.S. Cox, R.B. Croteau, D.M. Crothers, P.J. Crutzen, G.C. Daily, G.B. Dalrymple, J.L. Dangl, S.A. Darst, D.R. Davies, M.B. Davis, P.V. De Camilli, C. Dean, R.S. DeFries, J. Deisenhofer, D.P. Delmer, E.F. Delong, D.J. Derosier, T.O. Diener, R. Dirzo, J.E. Dixon, M.J. Donoghue, R.F. Doolittle, T. Dunne, P.R. Ehrlich, S.N. Eisenstadt, T. Eisner, K.A. Emanuel, S.W. Englander, W.G. Ernst, P.G. Falkowski, G. Feher, J.A. Ferejohn, A. Fersht, E.H. Fischer, R. Fischer, K.V. Flannery, J. Frank, P.A. Frey, I. Fridovich, C. Frieden, D.J. Futuyma, W.R. Gardner, C.J.R. Garrett, W. Gilbert, R.B. Goldberg, W.H. Goodenough, C.S. Goodman, M. Goodman, P. Greengard, S. Hake, G. Hammel, S. Hanson, S.C. Harrison, S.R. Hart, D.L. Hartl, R. Haselkorn, K. Hawkes, J.M. Hayes, B. Hille, T. Hôkfelt, J.S. House, M. Hout, D.M. Hunten, I.A. Izquierdo, A.T. Jagendorf, D.H. Janzen, R.Jeanloz, C.S. Jencks, W.A. Jury, H.R. Kaback, T. Kailath, P. Kay, S.A. Kay, D. Kennedy, A. Kerr, R.C. Kessler, G.S. Khush, S.W. Kieffer, P.V. Kirch, K. Kirk, M.G. Kivelson, J.P. Klinman, A. Klug, L. Knopoff, H. Kornberg, J.E. Kutzbach, J.C. Lagarias, K. Lambeck, A. Landy, C.H. Langmuir, B.A. Larkins, X.T. Le Pichon, R.E. Lenski, E. B. Leopold, S.A. Levin, M. Levitt, G. E. Likens, J. Lippincott-Schwartz, L. Lorand, C.O. Loverjoy M. Lynch, A.L. Mabogunje, T.F Malone, S. Manabe, J. Marcus, D.S. Massey, J.C. McWilliams, E. Medina, H.J. Melosh, D.J. Meltzer, C.D. Michener, E.L. Miles, H.A. Mooney, P.B. Moore, F.M.M. Morel, E.S. Mosley-Thompson, B. Moss, W.H. Munk, N. Myers, G.B. Nair, J. Nathans, E.W. Nester, R.A. Nicoll, R.P. Novick, J.F. O'Connell, P.E. Olsen, N.D. Opdyke, G.F. Oster, E. Ostrom, N.R. Pace, R.T. Paine, R.D. Palmiter, J. Pedlosky, G.A. Petsko, G.H. Pettengill, S.G. Philander, D.R. Piperno, T.D. Pollard, P.B. Price jr., P.A. Reichard, B.F. Reskin, R.E. Ricklefs, R.L. Rivest, J.D. Roberts, A.K. Romney, M.G. Rossmann, D.W. Russell, W.J.

Rutter, J.A. Sabloff, R.Z. Sagdeev, M.D. Sahlins, A.Salmond, J.R. Sanes, R. Schekman, J. Schellnhuber, D.W. Schindler, J. Schmitt, S.H. Schneider, V.L. Schramm, R.R. Sederoff, C.J. Shatz, F. Sherman, R.L. Sidman, K. Sieh, E.L. Simons, B.H. Singer, M.F. Singer, B. Skyrms, N.H. Sleep, B.D. Smith, S.H. Snyder, R.R. Sokal, C.S. Spencer, T.A. Steitz, K.B. Strier, T.C. Südhof, S.S. Taylor, J. Terborgh, D.H. Thomas, L.G. Thompson, R.T.T. Jian, M.G. Turner, S. Uyeda, J.W. Valentine, J.S. Valentine, J.L. Van Etten, K.E. Van Holde, M. Vaughan, S. Verba, P.H. Von Hippel, D.B. Wake, A. Walker, J.E. Walker, E.B. Watson, P.J. Watson, D. Weigel, S.R. Wessler, M.J. West-Eberhard, T.D. White, W.J. Wilson, R.V. Wolfenden, J.A. Wood, G.M. Woodwell, H.E. Wright jr., C. Wu, C. Wunsch, M.L. Zoback- 2010-Climate change and the integrity of science- Science 328: (#5979, 5/7) 689-690

Along with the greenhouse effect, rising atmospheric CO_2 concentrations is driving the **acidification of the oceans**, and this is expected to greatly alter ocean ecosystems and may drive many **coral reefs** to extinction.

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De´ath G., J.M. Lough, K.E. Fabricius- 2009-Declining coral calcification on the great barrier reef- Science 323: (#5910, 1/2) 116-119

Doney S.C- 2006-The dangers of ocean acidification- Scientific American 294: (#3, March) 58-65

Hoegh-Guldberg O., P.J. Mumby, A.J. Hooten, R.S. Steneck, P. Greenfield, E. Gomez, C.D. Harvell, P.F. Sale, A.J. Edwards, K. Caldeira, N. Knowlton, C.M. Eakin, R. Iglesias-Prieto, M. Muthíga, R.H. Bradbury, A. Dubi, M.E. Hatziolos- 2007-Coral reefs under rapid climate change and ocean acidification- Science 318: (#5857, 12/14) 1737-1742

Iglesias-Rodriguez M.D., P.R. Halloran, R.E.M. Rickaby, I.R. Hall, E. Calmenera-Hidalgo, J.R. Gittins, D.R.H. Green, T. Tyrrell, S.J. Gibbs, P. von Dassow, E. Rehm, E.V. Armbrust, K.P. Boessenkool- 2008-Phytoplankton calcification in a high-CO_2 world- Science 320: (#5874, 4/18) 336-340

Kintisch E., E. Stokstad- 2008-Ocean CO_2 studies look beyond coral- Science 319: (#5866, 2/22) 1029

Stone R- 2007-A world without corals?- Science 316: (5/4) 678-681

Yamamoto-Kawai M., F.A. McLaughlin, E.C. Carmack, S. Nishino, K. Shimada- 2009-Aragonite under saturation in the arctic ocean: Effects of ocean acidification and sea ice melt- Science 326: (#5956, 11/20) 1098-1100

Zeebe R.E., J.Z Zachos, K. Caldeira, T. Tyrrell- 2008-Carbon emissions and acidification- Science 321: (5885, 7/4) 51-52

Pollution of the oceans has expanded the **dead zone** areas, low in oxygen, in the oceans.

Diaz R.J., R. Rosenberg- 2008-Spreading dead zones and consequences for marine ecosystems- Science 321: (#5891, 6/15) 926-929

Stramma L., G.C. Johnson, J. sprintall, V. Mohrholz- 2008- Expanding ocean-minimum zones in the tropical oceans- Science 320: (#5876, 5/2) 655-658

How much could **sea levels** change? One way to assess this is to consider that in the past sea levels did reach 250 feet higher than today. Another is to model the changes based on more recent data.

Meier M.F., M.B. Dyurgerov, V.K. Rick, S. O'Neal, W.T. Pfetter, R.S. Anderson, S.P. Anderson, A.F. Glazovsky-   2007-Glaciers dominate eustatic sea-level rise in the 21st century- Science 317: (#5841, 8/24) 1064-1067

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Rahmstrof S- 2007-A semi-empirical approach to projecting future sea-level rise- Science 315: (1/19) 368-370

Shepherd A., D. Wingham- 2007-Recent sea-level contributions of the antarctic and greenland ice sheets- Science 315: (3/16) 1529-1532

The **ozone depletion** has been slowed, perhaps halted, but it has not yet been reversed.

Bassman J.H- 2004-Exosystem consequences of enhances solar ultraviolet radiation: Secondary plant metabolites as mediators of multiple trophic interactions in terrestrial plant communities- Photochemistry and Photobiology 79: (#5) 382-398

Kintisch E- 2007-Tougher ozone accord also addresses global warming- Science 317: (#5846, 9/28) 1843

Krizek D.T., W. Gao- 2004-Ultraviolet radiation and terrestrial ecosystems- Photochemistry and Photobiology 79: (#5) 379-381

Krizek D.T- 2004-Influence of PAR and UV-A in determining plant sensitivity and photomorphogenic responses to UV-B radiation- Photochemistry and Photobiology 79: (#4) 307-315

Lubin D., K.R. Arrigo, G.L. van Kijken- 2004-Increased exposure of southern ocean phytoplankton to ultraviolet radiation- Geophysical Research Letters 31: L09304

von Hobe M- 2007-Revisiting ozone depletion- Science 318: (#5858, 12/21) 1878-1879

Extinctions have occurred, and the **extinction rate** is rising. The consequences for attempts to preserve biodiversity may be severe.

Becker C.G., C.R. Fonseca, C.F.B. Haddad, R.F. Batista, P.I. Prado- 2007-Habitat split and the global decine of amphibians- Science 318: (#5857, 12/14) 1775-1777

Cardillo M., E.M. Mace, K.E. Jones, J. Bielby, O.R.P. Binida-Emonds, W. Sechrest, C.D.L. Orma, A. Purvis- 2005-Mulitple causes of high extinction risk in large mammal species- Science 309: (8/19) 1239-1241

Ceballos G., P.R. Ehrlich, J. Soberón, I. Salazar, J.P. Fay- 2005-Global mammal conservation: What must we manage?- Science 309: (7/22) 603-607

Daily G.C., R. Howarth, D.A. Vaccari, A.C. Morris, E.F. Lambin, S.C. Doney, P.H. Gleick, D.H. Fahey- 2010-Solutions to environmental threats- Scientific American 302: (#4, April) 58-60

Gill J.L., J.W. Williams, S.T. Jackson, K.B. Lininger, G.S. Robinson- 2009-Pleistocene megafaunal collapse, novel plant communities, and enhanced fire regimes in North America- Science 326: (#5956, 11/20) 1100-1103

Huey R.B., J.B. Losos, C. Moritz- 2010-Are lizards toast?- Science 328: (#5980, 5/14) 832-833

Kerr R.A- 2008-Experts find no evidence for a mammoth-killer impact- Science 319: (#5868, 3/7) 1331-1332

Pimm S.L., C. Jenkins- 2005-Sustaining the variety of life- Scientific American 293: (#3, Sept) 66-73

Schipper J., J.S. Chanson, F. Chiozza, N.A. Cox, M. Hoffmann, V. Katariya, J.J. Lamoreux, A.S.L. Rodrigues, S.N. Stuart, H.J. Temple, J. Baillie, L. Boitani, T.E. Lacher Jr., R.A. Mittermeier, A.T. Smith, D. Absolon, J.M. Aguiar, G. Amori, N. Bakkour, R. Baldi, R.J. Berridge, J. Bielby, P.A. Black, J.J. Blanc, T.M. Brooks, J.A. Burton, T.M. Butynski, G. Catullo, R. Chapman, Z. Cokeliss, B. Collen, J. Conroy, J.G. Cooke, G.A.B. da Fonseca, A.E. Derocher, H.T. Dublin, J.W. Duckworth, L. Emmons, R.H. Emslie, M. Festa-Bianchet, M. Foster, S. Foster, D.L. Garshelis, C. Gates, M. Gimenez-Dixon, S. Gonzalez, J.F. Gonzalez-Maya, T.C. Good, G. Hammerson, P.S. Hammond, D. Happold, M. Happold, J. Hare, R.B. Harris, C.E. Hawkins, M. Haywood, L.R. Heaney, S. Hedges, K.M. Helgen, C. Hilton-Taylor, S.A. Hussain, N. Ishii, T.A. Jefferson, R.K.B. Jenkins, C.H. Johnston, M. Keith, J. Kingdon, D.H. Knox, K.M. Kovacs, P. Langhammer, K. Leus, R. Lewison, G. Lichtenstein, L.F. Lowry, Z. Macavoy, G.M. Mace, D.P. Mallon, M. Masi, M.W. McKnight, R.A. Medellín, P. Medici, G. Mills, P.D. Moehlman, S. Molur, A. Mora, K. Nowell, J.F. Oates, W. Olech, W.R.L. Oliver, M. Oprea, B.D. Patterson, W.F. Perrin, B.A. Polidoro, C. Pollock, A. Powel, Y. Protas, P. Racey, J. Ragle, P. Ramani, G. Rathbun, R.R. Reeves, S.B. Reilly, J.E. Reynolds III, C. Rondinini, R.G. Rosell-Ambal, M. Rulli, A.B. Rylands, S. Savini, C.J. Schank, W. Sechrest, C. Self-Sullivan, A. Shoemaker, C. Sillero-Zubiri, N. De Silva, D.E. Smith, C. Srinivasulu, P.J. Stephenson, N. van Strien, B.K. Talukdar, B.L. Taylor, R. Timmins, D.G. Tirira, M.F. Tognelli, K. Tsytsulina, L.M. Veiga, J-C. Vié, E.A. Williamson, S.A. Wyatt, Y. Xie, B.E. Young- 2008-The status of the world's land and marine mammals: Diversity,

threat, and knowledge- Science 322: (#5899, 10/10) 225-230

Sinervo B., F. Méndez-de-la-Cruz, D.B. Miles, B. Heulin, E. Bastiaans, M. Villagrán-Santa Cruz, R. Lara-Resendiz, N. Martínez-Méndez, M.L. Calderón-Espinosa, R.N. Meza-Lázaro, H. Gadsden, L.J. Avila, M. Morando, I.J. De la Riva, P.V. Sepulveda, C.F.D. Rocha, N. Ibargüengoytía, C.A. Puntriano, M. Massot, V. Lepetz, T.A. Oksanen, D.G. Chapple, A.M. Bauer, W.R. Branch, J. Clobert, J.W. Sites jr-2010-Erosion of lizard diversity by climate change and altered thermal niches- Science 328: (#5980, 5/14) 894-899

Stokstad E- 2009-Debate continues over rainforest fate - with a climate twist- Science 323: (#5913, 1/23) 448

Stokstad E- 2005-Global analyses reveal mammals facing risk of extinction- Science 309: (7/22) 546-547

Stone R- 2007-The last of the leviathans- Science 316: (#5832, 6/22) 1684-1688

Zimmer C- 2007-Predicting oblivion: Are existing models up to the task?- Science 317: (#5840, 8/17) 892-893

The extent of **human impact** on the planet and ecosystems are many and varied. Here are a few examples.

Andreae M.O- 2007-Aerosols before pollution- Science 315: (1/5) 50-51

Barrett M.A., J.L. Brown, M.K. Morikawa J-N. Labat, A.D. Yoder- 2010-CITES designation for endangered rosewood in Madagascar- Science 328: (#5982, 5/28) 1109-1110

Bohannon J- 2010-The Nile delta's sinking future- Science 327: (#5972, 3/19) 1444-1447

Bohannon J- 2010-Madagascar's forests get a reprieve - but for how long?- Science 328: (#5974, 4/2) 23-24

Barnett T.P., D.W. Pierce, H.G. Hidalgo, C. Bonfils, B.D. Santer, T. Das, G. Bala, A.W. Wood, T. Nozawa, A.A. Mirin, D.R. Cayan, M.D. Dettinger- 2008-Human-induced changes in the hydrology of the Western United States- Science 319: (#5866, 2/22) 1080-1083

Diamond J- 2007-Easter Island reconsidered- Science 317: (#5845, 9/21) 1692-1694

French C- 2010-People, societies, and landscapes- Science 328: (#5977, 4/23) 443-444

Foley J.A., R. Defries, G.P. Asuer, C. Barford, G. Bonar, S.R. Carpenter, F.S. Chapin, M.T. Coe, G.C. Daily, H.K. Gibbs, J.H. Helkowski, T. Holloway, E.A. Howard, C.J. Kucharik, C. Monfreda, J.A. Patz, I.C. Prentice, N. Ranankutty, P.K. Snyder- 2005- Global consequences of land use- Science 309: (7/22) 570-574

Forest Certification Resource Center- 2004-World Hunger for Wood- Scientific American 250: (#6) 258

Grimm N.B., S.H. Faeth, N.E. Golubiewski, C.L. Redman, J. Wu, X. Bai, J.M. Briggs- 2008-Global change and the ecology of cities- Science 319: (#5864, 2/8) 756-760

Halfar J., R.M. Fujita- 2007-Danger of deep-sea mining- Science 316: (5/18) 987

Halpern B.S., S. Walbridge, K.A. Selkoe, C.V. Kappel, F. Micheli, C. D'Agrosa, J.F. Bruno, K.S. Casey, C. Ebert, H.E. Fox, R. Fujita, D. Heinemann, H.S. Lenihan, E.M.P. Madin, M.T. Perry, E.R. Selig, N. Spalding, R. Steneck, R. Watson-

2008-A global map of human impact on marine ecosystems- Science 319: (#5865, 2/15) 948-952

Inman M- 2009-Hot, flat, crowded - And preparing for the worst- Science 326: (#5953, 10/30) 662-663

Kaiser J- 2007-Canadian study reveals new class of potential POPs- Science 317: (#5835, 7/13) 182-183

Kerr R.A- 2007-Pollutant hazes extend their climate-changing reach- Science 315: (3/2) 1217

Laporte N.T., J.A. Stabach, R. Grosch, T.S. Lin, S.J. Goetz- 2007-Expansion of industrial logging in central Africa- Science 316: (6/8) 1451

Lohman D.J., D. Bickford, N.S. Sodhi- 2007-The burning issue- Science 315: (4/20) 376

McConnell J.R., R. Edwards, G.L. Kok, M.G. Flanner, C.S. Zender, E.S. Saltzman, J.R. Banta, D.R. Pasterls, M.M. Carter, J.D.W. Kahl- 2007-20th-century industrial black carbon emissions altered arctic climate forcing- Science 317: (#5843, 9/7) 1381-1384

Montgomery D.R- 2008-Dreams of natural streams- Science 319: (#5861, 1/18) 291-292

Morell V- 2007-Killing whales for science?- Science 316: (4/27) 532-534

Normile D- 2007-Getting at the roots of killer dust storms- Science 317: (#5836, 7/20) 314-316

Ruddiman W.F- 2005-How did humans first alter global climate?- Scientific American 292: (#3) 46-53

Service R.F- 2007-Delta blues, California style- Science 317: (#5837, 7/27) 442-445

Szidat S- 2009-Sources of asian haze- Science 323: (#5913, 1/23) 470-471

Walter R.C., D.J. Merrits- 2008-Natural streams and the legacy of water-powered mills- Science 319: (#5861, 1/18) 299-304

Watts R.D., R.W. Compton, J.H. McCammon, C.L. Rich, S.M. Wright, T. Owens, D.S. Ouren- 2007-Roadless space of the conterminous United States- Science 316: (5/4) 736-738

Worm B., E.B. Barbier, N. Beaumont, J.E. Dufty, C. Folke, B.S. Halpern, J.B.C. Jackson, H.K. Lotze, F. Micheli, S.R. Palumbi, E. Sala, K.A. Selkoe, J.J. Stachowicz, R. Watson- 2006-Impacts of biodiversity loss on ocean ecsytem services- Science 314: (11/3) 787-790

The growth of **human population** is driving many of the pressures we put on the environment.

Campbell M., J. Cleland, A. Ezeh, N. Prata- 2007-Return of the population growth factor- Science 315: (3/16) 1501-1502

Cohen J.E- 2005-Human population grows up- Scientific American 293: (#3, Sept) 48-55

Mace R- 2008-Reproducing in cities- Science 319: (#5864, 2/8) 764-766

Musser G- 2005-The climax of humanity- Scientific American 293: (#3, Sept) 44-47

In an ironic twist, because **protected areas** are of higher quality, they tend to attract humans to live near them putting even more pressure on conservation attempts.

Wittemyer G., P. Elsen, W.T. Bean, A.C.O. Burton, J.S. Brashares- 2008-Accelerated human population growth at protected area edges- Science 321: (#5885, 7/4) 123-126

BIO 108   2010
Homework set #1   Name: _____
Due 9:00 am, Monday, July 19. (Text references are indicated for each question.)
        Points earned: _____ = _____ for course.
                                  5
_____
1.) (8 pts) (Lecture 1. See pages 610-612, and figures 29.5 and 13.6, of the text.)

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The above represents the life cycle of a typical fern plant. Indicate specific examples of each of the
following in it by drawing a line to each example and labeling it with the appropriate letter.

        a.) Two cells just before fertilization will occur.
        b.) The first haploid (1N) cells in this life cycle.
        c.) A haploid multicellular generation.
        d.) A gamete made by mitosis.
        e.) Two points in this life cycle where sexual reproduction occurs.

Describe one major feature seen in the above life cycle but that is lacking from the poor deprived life
cycle of animals.

2.) (7 pts.) (Lecture 2. See chapter 12 of our text.)
Answer the following questions.
a.) What type of cytoskeletal structure is typically needed to acheive proper segregation of the sets of chromosomes during mitosis?

_____

b.) For either a typical eukaryotic cell **with**, or **without**, a cell wall (circle your choice) what type of cytoskeletal element is needed to carry out cytokinesis?

_____

c.) Describe the major difference between what is done by cytokinesis, versus what is done by mitosis, as two parts of the M-phase of the cell cycle.

d.) Identify a typical example for each of the following:

The first event of prometaphase:	
The last event of metaphase:	
The first event of anaphase:	

3.) (9 pts) (Lecture 3. See pgs. 253-258, 262-271.)
Consider three genes (Z, W, and T) in a hypothetical fish, and assume that these genes and their alleles behave in a way consistent with Mendel's findings.
a.) If a cell in a fish that is heterozygous for two of these unlinked genes and homozygous for the third undergoes meiosis, how many possible genotypic combinations will be expected to result?

_____

b.) If a test cross is to be done, what **must** be the genotype of one of the fish engaged in this cross?

_____

What are two possible genotypes of two individual fish who differ in genotype, but are similar in phenotype, from the situation given in part (a)

_____   _____

c.) If in the heterozygous fish given in item (a) there is independent assortment done between each of these three genes during meiosis I and the rest of meiosis I proceeds normally to make two daughter cells. But during meiosis II one of these daughter cells has a failure of segregation such that at the end of anaphase II all the chromosomes end up in one cell, then:

What will be the ploidy of that final cell? _____

Will these chromosomes be double- or single- chromatid chromosomes? _____

What is a likely genotype of this cell? _____

4.) (6 pts) (Lecture 4. See pgs. 271-281.)
This figure shows the results of a cross between two parent mice.

a.) What was the cross? (Indicate the genotypes of each parent in your answer.)

b.) In the biochemical pathway to produce the fur colors seen in these mice which gene's product acts earlier in the pathway?

c.) What is the best way to describe the relationship between the "B" and "b" alleles?

d.) What is the best way to decribe the relationship between the "B" and "C" genes?

e.) What is one way that these data are consistent with Mendelian genetics?

Sperm

¼ BC   ¼ bC   ¼ Bc   ¼ bc

Eggs

¼ BC   BBCC   BbCC   BBCc   BbCc

¼ bC   BbCC   bbCC   BbCc   bbCc

¼ Bc   BBCc   BbCc   BBcc   Bbcc

¼ bc   BbCc   bbCc   Bbcc   bbcc

9/16   3/16   4/16

3

5.) (6 pts) (Lecture 5. See pages 288-292 of our text.)
The original white-eyed fruit fly that Morgan's group studied helped to determine that the recessive
mutant allele for eye color was on the X
chromosome but not the Y. What would have
been the outcomes in the F1 and F2 generations
of the crosses they did, if instead this mutant
allele was dominant, occurred on both X and Y
chromosomes, and that the mutant allele was
initially on the Y chromosome of the white eyed
male fly? (6 pts)

a.) Outline your thinking by noting the locations
of the wildtype allele (w+), and mutant allele (w)
on each of the chromosomes shown in the crosses
shown the figure. Also in each box of the two
Punnett squares indicate if the individual will
have white or red eyes.

b.) What would this type of sex linkage predict
about that offspring that would be different from
what would be expected from sex linkage on only
the X chromosome?

6.) (7 pts) (Lecture 6. See chapter 31 of our text.)
In the figure given below indicate a correct location of each of the following items by drawing a line to the item and labeling it
with the appropriate letter.

a.) An arrow in the figure that indicates the occurrence of meiosis.
b.) A stage that is just after the completion of plasmogamy.
c.) A zygote.
d.) A spore.
e.) A dikaryotic cell.
f.) An ascus.

The fungus shown in this figure is a member of which phylum of fungi?

_____

7.) Answer each of the following questions. (7 pts) (Lecture 7. See pgs. 214-215, 305-310.)
a.) In Griffith's experiment, after treating mice with R-strain *Streptococcus pneumonae* mixed with heat-killed S-strain cells he isolated the bacteria from the dead mouse and then took the additional step of confirming that the bacteria were now S-strain cells. If he had not done this last step what would have been a likely alternative explanation to his conclusion of there being a transforming factor? [Hint: Consider this treatment relative to the heat-killed S-strain treatment.]

b.) In the study done by Hershey and Chase they assumed that proteins would not have the ^{32}P radioisotope associated with them. What class of enzymes phosphorylate proteins and what class of enzymes dephosphorylate proteins? What is the relevance of the existence of such enzymes to the conclusion reached by Hershey and Chase?

c.) Many proteins are known to bind to specific nucleotide sequences of DNA. Describe how an advocate of the proteins-as-genes hypothesis might use this information together with Chargaff's findings in an argument that would advocate for proteins as being the genetic material.

6

8.) (8 pts) (Lecture 8. See pgs. 311-319.)
In the figure shown here draw a line specifically to each item listed below and label it with the correct letter. Then answer the question below about the figure.

a.) The leading strand.
b.) The lagging strand.
c.) An Okazaki fragment.
d.) RNA.
e.) DNA ligase.
f.) DNA polymerase.
g.) A 5' end of a DNA strand.
As a whole this figure represents a structure called a: _____.

9.) (8 pts) (Lecture 9. See pgs. 331-336, and 364-366 of our text.)
Describe the major events that occur during each of the following processes, but not in the others:

Transcriptional Initiation:

RNA splicing:

RNA interference:

RNA export:

10.) (9 pts) (Lecture 10. See pgs. 328-331.)
The following hypothetical peptide is a product of an endoprotease acting to cleave certain sites in a larger polypeptide. You have isolated it and determined the following amino acid sequence for it:

(amino end of...)-THR-ASP-TRP-LEU-VAL-ASN-(... carboxyl end)

a.) What is one possible nucleotide sequence of RNA that would code for this peptide? Indicate the 5' and 3' ends of the RNA sequence you propose.

b.) For just the first codon (coding for THR) in the above peptide. What would be a likely sequence of the anticodon region on the THR-tRNA that should be used for this codon? Indicate the 5' and 3' ends of it.

c.) If the first nucleotide at the 5' end of your answer in (a) was ignored during translation, so that the reading frame were shifted over by one nucleotide, what then would be the resulting amino acid sequence in the peptide? Indicate the amino- and carboxyl- termini of your proposed peptide. (Obviously the last codon will only have two nucleotides, so ignore it for this question.)

11.) (9 pts) (Lecture 11. See pgs. 351-358, 362-366.)
Consider the following figure.
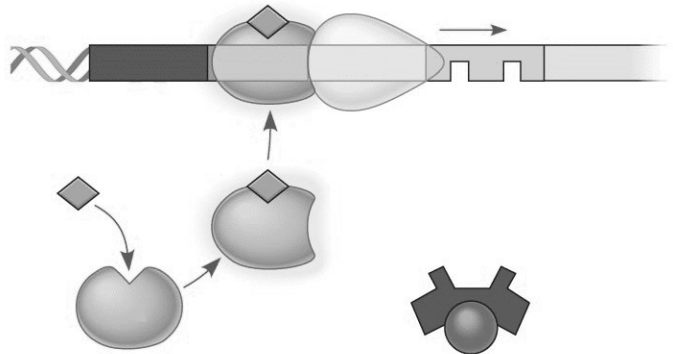
a.) This figure shows control of gene expression at what level?

_____

b.) Identify a type of control of gene expression that typically occurs before that shown in the figure.

c.) Identify a type of control of gene expression that typically occurs after the stage shown in this figure.

d.) Where possible, label each of the following in the figure by drawing a line to a specific example of it and indicating the approprate letter (if not possible, don't label it....   ;-).

      A.) Repressor protein.   B.) RNA polymerase.
      C.) DNA polymerase.   D.) CAP protein.
      E.) Operator region.   F.) Ubiquitin.

12.) (6 pts) (Lecture 12. See pgs. 256-362, 434-442.)
a.) What are two epigenetic changes that often influence the rate of eukaryotic gene expression?

b.) Often new gene copies are made in eukaryotes (gene copy number is known to vary between individual people). What is a potential benefit and what is a likely cost of duplication of the number of copies of a gene in our genome?   Describe the reasoning behind your answer in each case.

Benefit:

Cost:

BIO 108   2010

Proposed Answers for Homework set #1.

Below are some possible answers to the questions in this homework set. Please note that other answers might receive full or partial credit.

Please look over the proposed answers both so that you can gain a sense of where I was headed with each question, and to give yourself some feedback on the issues raised by these questions.

Anyone who wishes more feedback may come see me about items in this homework set. Please see the course syllabus for information about how to request regrading of any lecture item.

Homework set #1   Name: _____

Due 9:00 am, Monday, July 19. (Text references are indicated for each question.)

        Points earned: _____ = _____ for course.
                                    5

1.) (8 pts) (Lecture 1. See pages 610-612, and figures 29.5 and 13.6, of the text.)

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The above represents the life cycle of a typical fern plant. Indicate specific examples of each of the following in it by drawing a line to each example and labeling it with the appropriate letter.

        a.) Two cells just before fertilization will occur.
        b.) The first haploid (1N) cells in this life cycle.
        c.) A haploid multicellular generation.
        d.) A gamete made by mitosis.
        e.) Two points in this life cycle where sexual reproduction occurs.

Describe one major feature seen in the above life cycle but that is lacking from the poor deprived life cycle of animals.

   Plants use meiosis to make spores, while animals have a more limited life cycle that does not include spores.

2.) (7 pts.) (Lecture 2. See chapter 12 of our text.)
Answer the following questions.
a.) What type of cytoskeletal structure is typically needed to acheive proper segregation of the sets of chromosomes during mitosis?

microtubules

b.) For either a typical eukaryotic cell **with**, or **without**, a cell wall (circle your choice) what type of cytoskeletal element is needed to carry out cytokinesis?

microfilaments

c.) Describe the major difference between what is done by cytokinesis, versus what is done by mitosis, as two parts of the M-phase of the cell cycle.

Mitosis separates the genetic material in one original nucleus into two new nuclei, all within the same cell.
Cytokinesis takes the material in a cell and divides it up into two new separate daughter cells.

d.) Identify a typical example for each of the following:

The first event of prometaphase:

Nuclear envelope breaks down.

The last event of metaphase:

The chromosomes align at the metaphase plate.

The first event of anaphase:

The double-chromatid chromosomes are converted into single-chromatid chromosomes.

3.) (9 pts) (Lecture 3. See pgs. 253-258, 262-271.)
Consider three genes (Z, W, and T) in a hypothetical fish, and assume that these genes and their alleles behave in a way consistent with Mendel's findings.
a.) If a cell in a fish that is heterozygous for two of these unlinked genes and homozygous for the third undergoes meiosis, how many possible genotypic combinations will be expected to result?

4

b.) If a test cross is to be done, what **must** be the genotype of one of the fish engaged in this cross?

zz ww tt (a test cross has one individual who is homozygous recessive)

What are two possible genotypes of two individual fish who differ in genotype, but are similar in phenotype, from the situation given in part (a)

Zz Ww Tt   *ZZ WW TT*

c.) If in the heterozygous fish given in item (a) there is independent assortment done between each of these three genes during meiosis I and the rest of meiosis I proceeds normally to make two daughter cells. But during meiosis II one of these daughter cells has a failure of segregation such that at the end of anaphase II all the chromosomes end up in one cell, then:

What will be the ploidy of that final cell?   _Diploid_

Will these chromosomes be double- or single- chromatid chromosomes?   _Single_

What is a likely genotype of this cell?   _zz WW TT (There are many possiblities here.)_

4.) (6 pts) (Lecture 4. See pgs. 271-281.)
This figure shows the results of a cross between two parent mice.

a.) What was the cross? (Indicate the genotypes of each parent in your answer.)

 Bb Cc X Bb Cc

b.) In the biochemical pathway to produce the fur colors seen in these mice which gene's product acts earlier in the pathway?

 Gene C.

c.) What is the best way to describe the relationship between the "B" and "b" alleles?

 Allele B is dominant to allele b.

d.) What is the best way to decribe the relationship between the "B" and "C" genes?

 Gene C has an epigenetic effect on gene B.

e.) What is one way that these data are consistent with Mendelian genetics?

 These genetic factors are inherited as distinct particles, which is consistent with Mendel's model.

Sperm

Eggs	$\frac{1}{4}$ BC	$\frac{1}{4}$ bC	$\frac{1}{4}$ Bc	$\frac{1}{4}$ bc
$\frac{1}{4}$ BC	BBCC	BbCC	BBCc	BbCc
$\frac{1}{4}$ bC	BbCC	bbCC	BbCc	bbCc
$\frac{1}{4}$ Bc	BBCc	BbCc	BBcc	Bbcc
$\frac{1}{4}$ bc	BbCc	bbCc	Bbcc	bbcc

$\frac{9}{16}$ $\frac{3}{16}$ $\frac{4}{16}$

3

5.) (6 pts) (Lecture 5. See pages 288-292 of our text.)
The original white-eyed fruit fly that Morgan's group studied helped to determine that the recessive mutant allele for eye color was on the X chromosome but not the Y. What would have been the outcomes in the F1 and F2 generations of the crosses they did, if instead this mutant allele was dominant, occurred on both X and Y chromosomes, and that the mutant allele was initially on the Y chromosome of the white eyed male fly? (6 pts)

a.) Outline your thinking by noting the locations of the wildtype allele (w+), and mutant allele (w) on each of the chromosomes shown in the crosses shown the figure. Also in each box of the two Punnett squares indicate if the individual will have white or red eyes.

b.) What would this type of sex linkage predict about the offspring that would be different from what would be expected from sex linkage on only the X chromosome?

When the gene is only on the X chromosome then it is possible for some of the F2 females to have white eyes. But when the mutant allele is on the Y chromosome, and dominant, then it is not possible for any female flies to have white eyes.
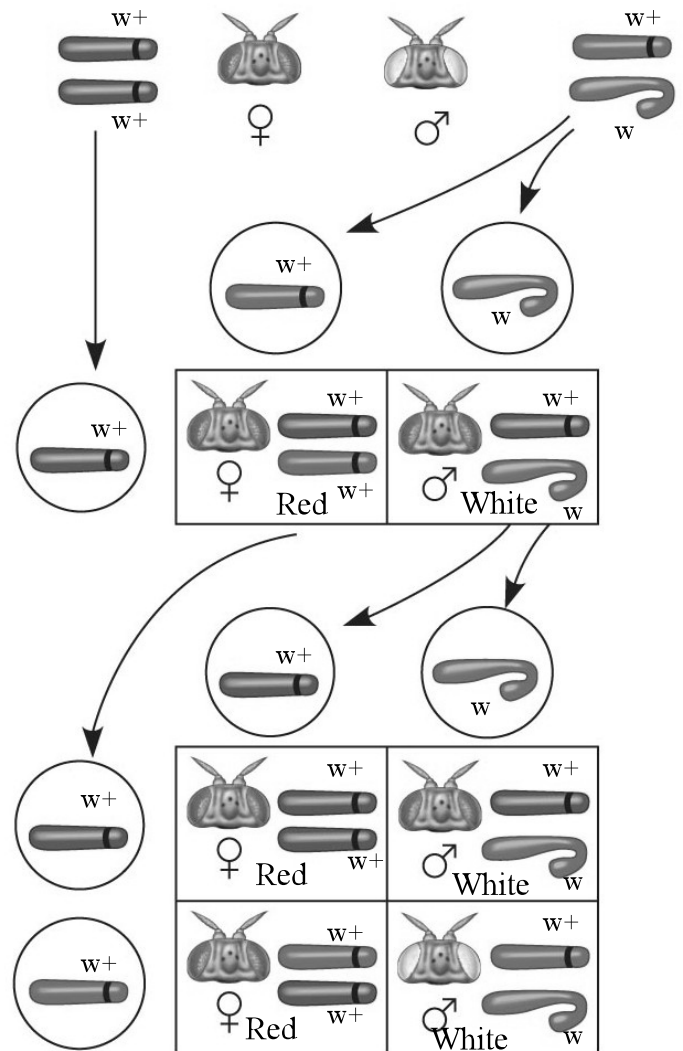
6.) (7 pts) (Lecture 6. See chapter 31 of our text.)
In the figure given below indicate a correct location of each of the following items by drawing a line to the item and labeling it with the appropriate letter.

a.) An arrow in the figure that indicates the occurrence of meiosis.
b.) A stage that is just after the completion of plasmogamy.
c.) A zygote.
d.) A spore.
e.) A dikaryotic cell.
f.) An ascus.

The fungus shown in this figure is a member of which phylum of fungi?

Ascomycota
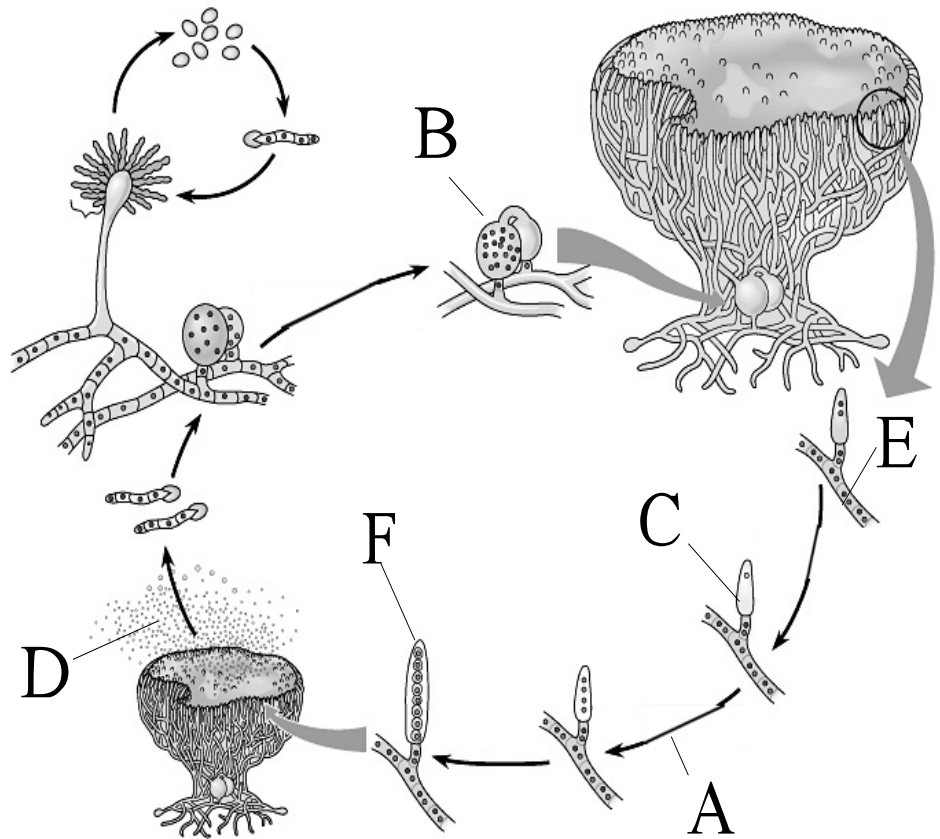
7.) Answer each of the following questions. (7 pts) (Lecture 7. See pgs. 214-215, 305-310.)
a.) In Griffith's experiment, after treating mice with R-strain *Streptococcus pneumonae* mixed with heat-killed S-strain cells he isolated the bacteria from the dead mouse and then took the additional step of confirming that the bacteria were now S-strain cells. If he had not done this last step what would have been a likely alternative explanation to his conclusion of there being a transforming factor? [Hint: Consider this treatment relative to the heat-killed S-strain treatment.]

If there was some factor in the dead S-strain extracts that needed to be converted to a toxic state by live R-stain cells, then the R-stain cells would not be transformed to S-strain cells, but the mouse would still be killed. To refute this possiblity Griffith had to isolate the bacteria and confirm that it had been transformed.

b.) In the study done by Hershey and Chase they assumed that proteins would not have the ^{32}P radioisotope associated with them. What class of enzymes phosphorylate proteins and what class of enzymes dephosphorylate proteins? What is the relevance of the existence of such enzymes to the conclusion reached by Hershey and Chase?

Protein kinases phosphorylate proteins, and phosphatases remove the phosphates. Thus in a living cell there will be some proteins with phosphates attached to them, and some of those phosphates should be the ^{32}P. Thus this isotope is not a perfect label for just the nucleic acids. It is possible that some of the ^{32}P is attached to proteins.

c.) Many proteins are known to bind to specific nucleotide sequences of DNA. Describe how an advocate of the proteins-as-genes hypothesis might use this information together with Chargaff's findings in an argument that would advocate for proteins as being the genetic material.

If the DNA sequence and base proportions differ between species, then the types of proteins that bind with this DNA should also differ. Since we would expect different genes to exist in different species, these genes could be proteins that bind to the different DNA regions. Thus Chargaff's findings just removed the steriotype that DNA was a non-variable molecule, and did not really refute the possibility of proteins-as-genes.

8.) (8 pts) (Lecture 8. See pgs. 311-319.)
In the figure shown here draw a line specifically to each item listed below and label it with the correct letter. Then answer the question below about the figure.

a.) The leading strand.
b.) The lagging strand.
c.) An Okazaki fragment.
d.) RNA.
e.) DNA ligase.
f.) DNA polymerase.
g.) A 5' end of a DNA strand.
As a whole this figure represents a structure called a: *replication fork* .
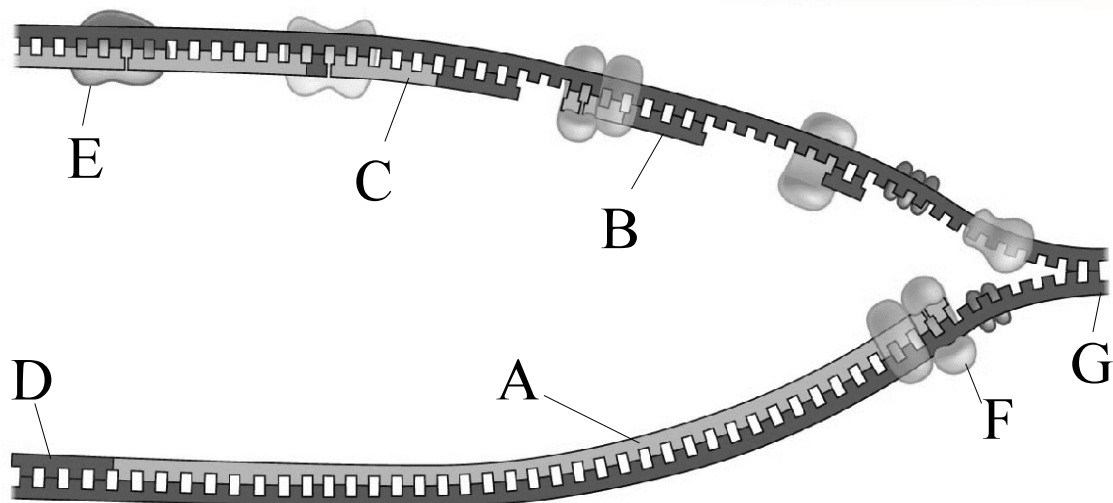
9.) (8 pts) (Lecture 9. See pgs. 331-336, and 364-366 of our text.)
Describe the major events that occur during each of the following processes, but not in the others:

Transcriptional Initiation: *The RNA polymerase binds at the promoter, and once it shifts to the start site it begins the formation of an RNA transcript.*

RNA splicing: *This removes introns and joins the ends of the neighboring exons in a primary RNA transcript.*

RNA interference: *Mature mRNA can be targeted by complementary RNAs that are bound to an RNAase which can then cut up the mRNA and prevent gene expression.*

RNA export: *RNA is physically moved from the nuclear space to the cytosolic space of the cell.*

10.) (9 pts) (Lecture 10. See pgs. 328-331.)
The following hypothetical peptide is a product of an endoprotease acting to cleave certain sites in a larger polypeptide. You have isolated it and determined the following amino acid sequence for it:
(amino end of...)-THR-ASP-TRP-LEU-VAL-ASN-(... carboxyl end)
a.) What is one possible nucleotide sequence of RNA that would code for this peptide? Indicate the 5' and 3' ends of the RNA sequence you propose.

5'- ACC - GAC - UGG - CUG - GUU - ACC - 3'

b.) For just the first codon (coding for THR) in the above peptide. What would be a likely sequence of the anticodon region on the THR-tRNA that should be used for this codon? Indicate the 5' and 3' ends of it.

3' - UGG - 5'

c.) If the first nucleotide at the 5' end of your answer in (a) was ignored during translation, so that the reading frame were shifted over by one nucleotide, what then would be the resulting amino acid sequence in the peptide? Indicate the amino- and carboxyl- termini of your proposed peptide. (Obviously the last codon will only have two nucleotides, so ignore it for this question.)

(N-terminal) - PRO - THR - GLY - TRP - LEU - (C-terminal)

11.) (9 pts) (Lecture 11. See pgs. 351-358, 362-366.)
Consider the following figure.
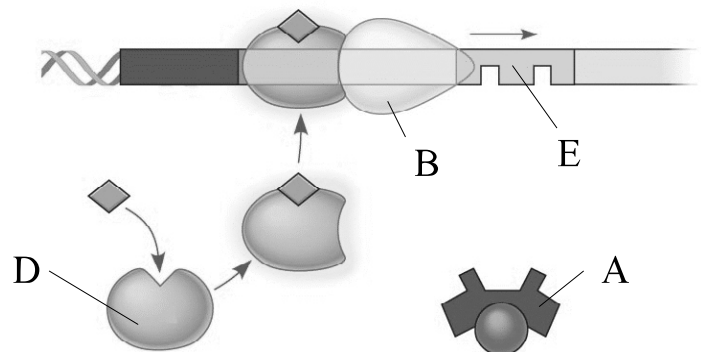
a.) This figure shows control of gene
expression at what level?

Transcriptional Level

b.) Identify a type of control of gene
expression that typically occurs before
that shown in the figure.

*Before this would be the supercoiling state of the DNA, which can alter the ability of the proteins
needed for transcription to bind well with the DNA.*

c.) Identify a type of control of gene expression that typically occurs after the stage shown in this
figure.

*After this there can be control over how stable the mRNA will be. It might be degraded rapidly,
or used for a long time, and this will alter the extent of gene expression.*

d.) Where possible, label each of the following in the figure by drawing a line to a specific example
of it and indicating the approprate letter (if not possible, don't label it....   ;-).

    A.) Repressor protein.   B.) RNA polymerase.
    C.) DNA polymerase.   D.) CAP protein.
    E.) Operator region.   F.) Ubiquitin.

12.) (6 pts) (Lecture 12. See pgs. 256-362, 434-442.)
a.) What are two epigenetic changes that often influence the rate of eukaryotic gene expression?

Methylation of DNA, and acetylation of histones.

b.) Often new gene copies are made in eukaryotes (gene copy number is known to vary between individual people). What is a potential benefit and what is a likely cost of duplication of the number of copies of a gene in our genome? Describe the reasoning behind your answer in each case.

 Benefit:

If a fast response is needed, and a fast rate of gene expression is called for, then having many copies of the gene means that once allowed to begin expression there will be alot more mRNA made in a given period of time than could be made with just one copy of the gene.

 Cost:

To make the extra DNA for these gene copies takes carbon, nitrogen and phosphates that have to be obtained, and production of this DNA costs energy. Thus accumulation of alot of extra DNA can slow the growth rate of the organism.

Homework set #2   Name: _____

Due 9:00 am, Tuesday, July 27. (Text references are indicated for each question.)

      Points earned: _____ = _____ for course.

                  5

_____

1.) (10 pts) (Lecture 13. See pgs. 381-390 of our text.)

Answer the following questions related to the figure at the right.

a.) Identify the letter of the stage that best matches each of the following descriptions.

_____ A restriction enzyme acts on the cell's DNA.

_____ Gene expression by the viral genes occurs.

_____ Receptors of the virus bind to cell-specific surface factors.

_____ A stage in which there is NO expression of viral genes.

b.) Roughly, the length of the bacteriophage shown in stage A of this

figure is _____, while the length of a typical bacterial cell is

roughly _____.

A

E

B

C

D

c.) Is the viral cycle shown in this figure a lysogenic or a lytic cycle? Explain the reasoning behind your answer.

d.) What is one thing that a retrovirus does that the bacteriovirus shown in the figure does not do?

_____

e.) What is one thing that is done by both a retrovirus and this bacteriophage?
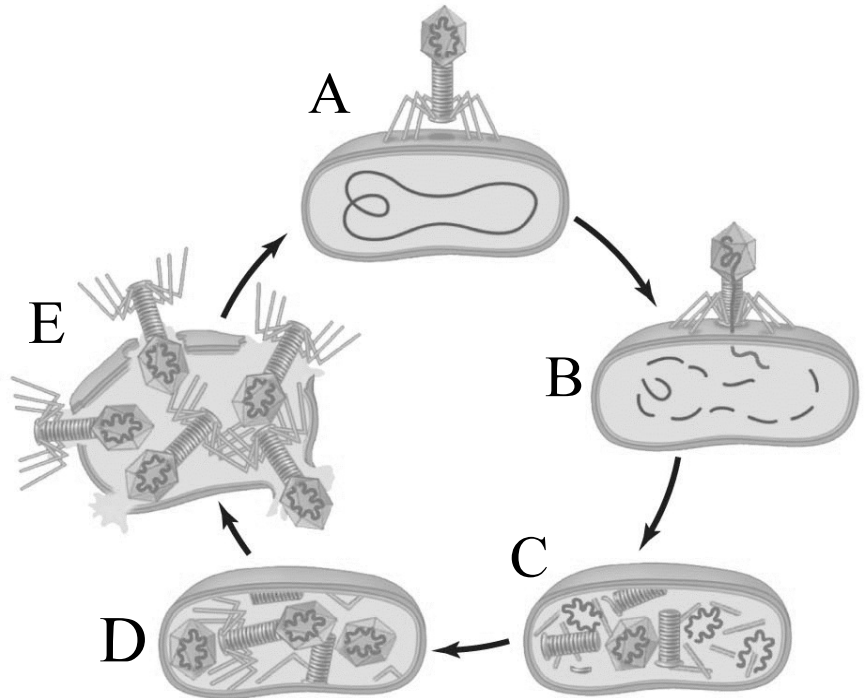
_____

2.) (9 pts.) (Lecture 14. See pgs. 561-564 of our text.)

Consider the processes of transduction and conjugation as seen in bacteria.

a.) Are the processes of transduction and conjugation best described as sexual or asexual processes? Describe the reasoning behind your answer.

b.) Which process, transduction or conjugation, is more likely to result in something favorable for the bacterium? Describe a major reason in support of your answer.

c.) Other than the use of DNA restriction and ligation, what is another feature of transduction and conjugation that is similar to crossing over that is so often seen during meiosis in eukaryotic cells?

d.) Is the process of transduction most likely to be beneficial to an individual bacterial cell or to the species as a whole? Support your answer by pointing out a specific benefit at the level you select.

3.) (6 pts) (Lecture 15. See pgs. 408-409.)
The approach to DNA sequencing given in fig. 20.12 shows the use of four distinct types of fluorescent tags so that each labeled ddNTP has a distinct color. When Sanger invented this method he did not use distinctly colored tags. Instead he ran the system with just one of each type of dideoxynucleotide at a time in it, meaning he had to run the system four times to fully sequence a template strand. Assume that the template strand shown in figure 20.12 was sequenced using Sanger's original method and using all the other materials shown in the figure. Fill in the table below to show the relative locations of the resulting bands of DNA in each of the appropriate lanes of a hypothetical electrophoretic gel.

	Lanes of Electrophoretic Gel			
(Wells of the gel)	Lane using tagged ddATP	Lane using tagged ddCTP	Lane using tagged ddTTP	Lane using tagged ddGTP
(Top of gel)				
(Bottom of gel)				

What would likely happen to the bands shown in the lane using tagged ddATP if the concentration of this dideoxynucleotide was a hundred fold higher than it should be when running the sequencing reaction?

4.) (10 pts) (Lecture 24. See pgs. 396-400.)

See fig. 20.4 in the text and answer the following questions about items shown in it.

a.) When the bacterial plasmid is cut by the restriction enzyme how many linear DNA pieces are created? How many "sticky ends" are present in each of these pieces?

DNA pieces:	Number of Sticky ends:

b.) In addition to the AmpR and lacZ genes, what other specific section of DNA would be very useful to have in this bacterial plasmid, and what is its role?

c.) Assuming, as shown in fig. 20.4, that the pieces from the bacterial plasmid are mixed with copies of the pieces of linear hummingbird DNA with similar sticky ends and ligated to form a new recombinant plasmid. Is it possible for such a recombinant plasmid, when in bacteria of a colony, to result in a phenotype of antibiotic resistance and a blue color when grown on the culture media used in step 5 of this figure? If not possible, explain why. If possible, diagram a plasmid that would be consistent with this outcome indicating the locations of the genes (or start and ending parts of the genes) lacZ and Amp^R, and humming bird DNA pieces in it.

d.) Given just the pieces from the bacterial plasmid (i.e. ignore any hummingbird DNA fragments) could a plasmid be made that would give a bacterial colony antibiotic resistance and have a white color when put on the culture media used in step 5 of this figure? If not possible, explain why. If possible, diagram a plasmid that would be consistent with this outcome indicating the locations of the genes (or start and ending parts of the genes) lacZ and Amp^R in the final plasmid.

5.) (6 pts) (Lecture 23. See pgs. 344-346, 356-363, 373-377.)
A change in nucleotide sequence in the coding region of a gene might cause a proto-oncogene to be converted into an oncogene, but what are three ways that heritable changes outside of a protein-coding region can also cause the activation of oncogenic activity? Describe for each change that you identify what it might do that could result ultimately in the induction of cancer.

6.) (9 pts) (Lecture 19. See chapter 43 of our text.)
Identify three types of receptors that occur in the mammalian immune system each of which detects non-self items. For each class of receptor that you note, indicate a specific cell type it is located in, and describe a major role it that cell type then plays in the action of immune system.

7.) (11 pts) (Lecture 16. See fig. 46.12 and pgs. 1021-1025.)
See the figure at the right in answering the following questions concerning animal gamete formation and fertilization.

a.) Which letter(s) indicate stages of:

        Dikaryotic cell(s):   _____
        Diploid cell(s):   _____
        Haploid cell(s):   _____

b.) Label with a line extending from the letter "P" the polar bodies in this figure.

c.) In addition to its nucleus, most animal sperm

    also contribute a _____ to the resulting zygote.

d.) In mammals, what specific structure typically contains the primary oocyte?

    _____

e.) Identify and describe the event that would normally occur next after stage "D" of this figure.

f.) What two receptors, located where and detecting what, are typically involved in many animals' fertilization to ensure species specificity?

7

Dorsal lip of blastopore

Pigmented gastrula (donor embryo)

Nonpigmented gastrula (recipient embryo)
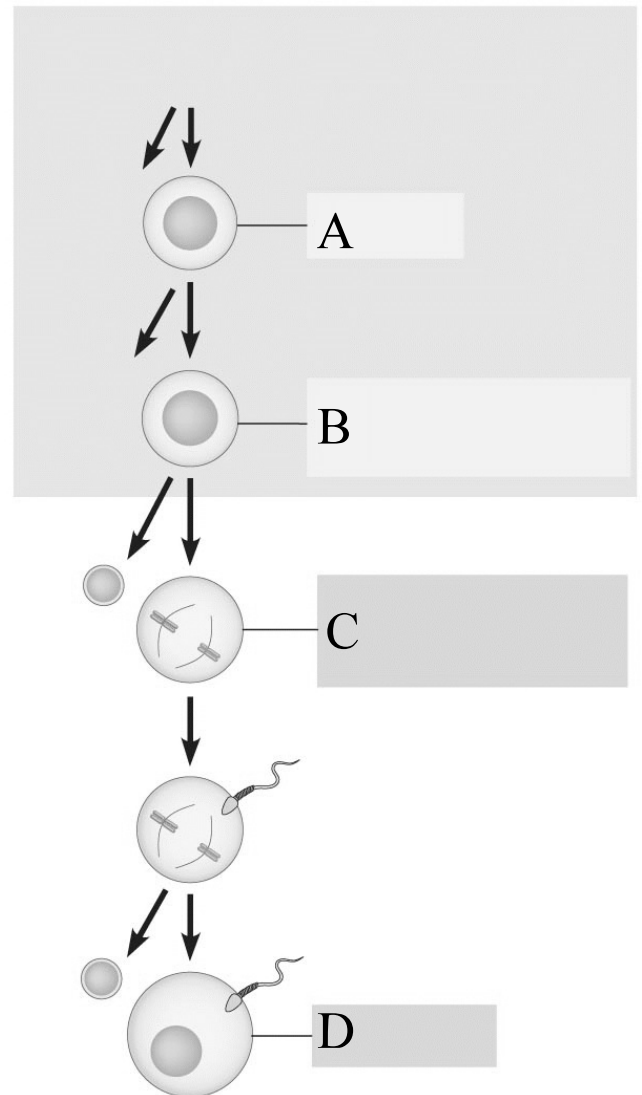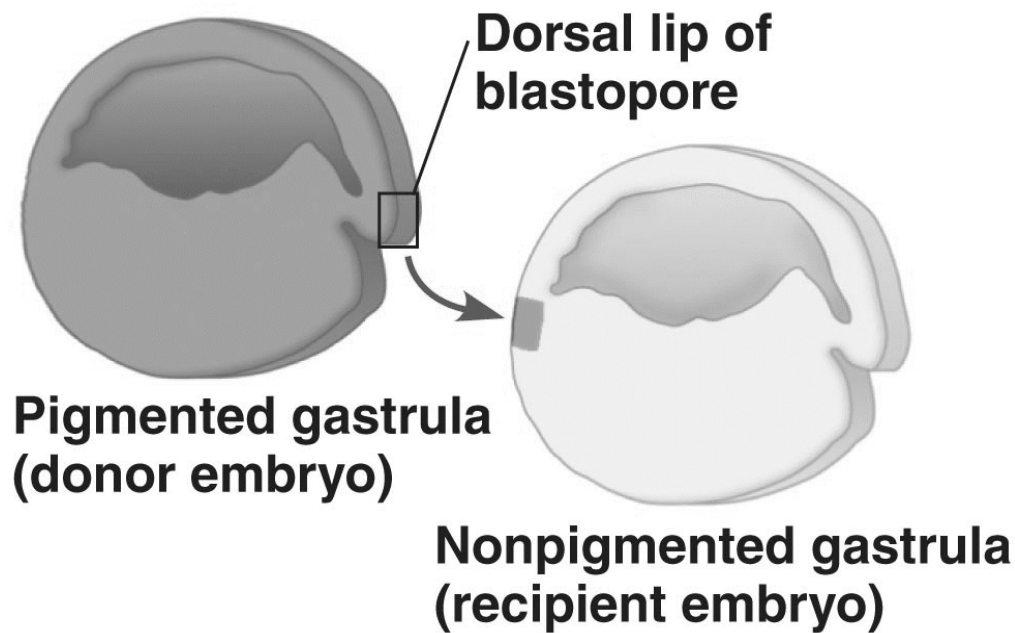
8.) (10 pts) (Lecture 20. See pgs. 1025-1042.)
Refer to the above images in answering the following questions.
a.) The above embryos are most likely from which type of animal? (Circle your choice.)

       bird   **amphibian**   **mammalian**
        Identify one feature visible in the above embryos that supports your answer, and describe
how it does so.

b.) Label an example of each of the following in the above embryos by drawing a line to it from
the appropriate number.
        1.) Blastocoel.   2.) Area likely to become nervous tissue.
        3.) Vegetal pole.   4.) Location of the future anus.
c.) Fill in the following blanks with the most appropriate term or phrase.
        The dark area transplanted to the pale gastrula will likely secrete items that inhibit

        _____. This will induce the process of _____ on that
side of the recipient embryo, and this may result in the formation of a second

        _____ organ system.

9.) (10 pts) (Lecture 21. See pgs. 223-225, 366-373)
Consider the following questions concerning determination and differentiation in development.
a.) What is one example of a so-called "master" regulatory gene?

_____

The above gene codes for a product that most likely must do what?

 If the product coded for by this gene was properly injected into a mature neuron, would this adult cell most likely take on a new phenotype or not? Explain the reasoning behind your answer.

b.) During the development of the frog secondary oocyte it receives *Vgl* RNA from the surrounding cells in the frog follicle. This *Vgl* RNA becomes localized at the vegetal pole as the oocyte matures. A recessive form of the gene for this RNA, *vgl*, has a single nucleotide change, but the RNA it codes for is unable to support subsequent normal embryo pattern formation. Assume that two frogs who are heterozygous for this gene (i.e. both are *Vgl vgl*) mate. What percentage of their tadpoles are likely to have abnormal embryonic development?

_____

What are two other items that are often sent by nurse/follicle cells to the developing oocyte of many animals?

c.) If in an animal cell the intracellular proteins Ced-4 and Ced-3 become activated, what is a likely final phenotype of this cell?

_____

Identify an example in a specific normal developmental pattern that uses this system.

10.) (8 pts) (Lecture 22. See chapter 26 and pg. 699.)
The following is a table of characteristics seen in several chordate species.

	Lizard	Lamprey	Salmon	Lancelet	Rabbit
Notochord	Yes	Yes	Yes	Yes	Yes
Vertebrae	Yes	Yes	Yes	No	Yes
Mammary Glands	No	No	No	No	Yes
Hinged Jaw	Yes	No	Yes	No	Yes
Four Limbs	Yes	No	No	No	Yes

a.) Based on the information in this table, which species would best serve as the out-group?

_____

b.) By what name are the species in the in-group typically called as a group?

_____

c.) Draw the most parsimonious cladogram possible based on the above data. In it, indicate the species, and the point of likely appearance of each characteristic.

d.) Identify two species from the table that if included with their common ancestor would make up a monophyletic group.

e.) Identify two species from the table that if included with their common ancestor would make up a paraphyletic group.

11.) (11 pts) (Lecture 17. See pgs. 625-628, and 801-811.)
Examine the figure shown at the right and answer the following questions about it.

a.) Label an example of each of the following
items by drawing a line to it with the appropriate
number.

    1.) An ovule.
    2.) A triploid (3N) region.
    3.) An integument.
    4.) A structure, as shown in the figure,
        capable of creating spores of some
        type without having to do a lot of
        growth first.
    5.) The wall of the ovary.

b.) The young plant shown to be growing in the
      middle of this figure is of which
      generation?

    _____

    It gets its food for growth from what
immediate source?

    _____

c.) When mature the structure that will surround
and include the mature ovule will best be called
a:

    _____
Describe a major function of this structure in the
life cycle of flowering plants.

12.) (9 pts) (Lecture 18. See pgs. 610-615.)
In the figure below indicate a location of an example of each of the following items by drawing a line to the item and labeling that line with the appropriate letter.
- a.) Sporophyte
- b.) Gametophyte
- c.) Sporangium
- d.) Archegonium
- e.) Antheridium
- f.) Zygote
- g.) Spore

Identify the structure(s) involved in sexual reproduction in the above figure, and note what each does that is sexual.

BIO 108 2010

Proposed Answers for Homework set #2.

Below are some possible answers to the questions in this homework set. Please note that other answers might receive full or partial credit.

Please look over the proposed answers both so that you can gain a sense of where I was headed with each question, and to give yourself some feedback on the issues raised by these questions.

Anyone who wishes more feedback may come see me about items in this homework set. Please see the course syllabus for information about how to request regrading of any lecture item.

BIO 108   2010
Homework set #2   Name: _____
Due 9:00 am, Tuesday, July 27. (Text references are indicated for each question.)
         Points earned: _____ = _____ for course.
                              5

1.) (10 pts) (Lecture 13. See pgs. 381-390 of our text.)
Answer the following questions
related to the figure at the right.

a.) Identify the letter of the stage that
best matches each of the following
descriptions.

   _B_   A restriction enzyme acts on
         the cell's DNA.
   _C_   Gene expression by the viral
         genes occurs.
   _A_   Receptors of the virus bind to
         cell-specific surface factors.
   _A_   A stage in which there is NO
         expression of viral genes.

b.) Roughly, the length of the
bacteriophage shown in stage A of
this

figure is   _230 nm_   , while the
length of a typical bacterial cell is

roughly   _2 μm_   .

c.) Is the viral cycle shown in this figure a lysogenic or a lytic cycle? Explain the reasoning
behind your answer.

 *Lytic. The infected cell proceeds directly to making and releasing viral particles. There is
 no quiescent stage as would be seen in a lysogenic cycle.*

d.) What is one thing that a retrovirus does that the bacteriovirus shown in the figure does not
do?
         It uses RNA as a template to guide the formation of new DNA.
e.) What is one thing that is done by both a retrovirus and this bacteriophage?

         Both viruses take over the cellular machinery of the host cell.
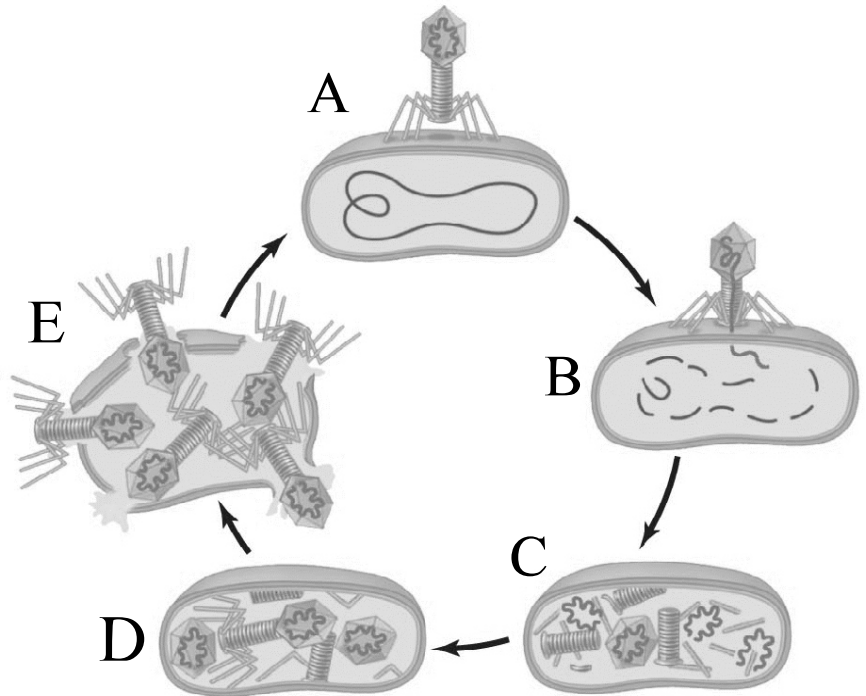
2.) (9 pts.) (Lecture 14. See pgs. 561-564 of our text.)
Consider the processes of transduction and conjugation as seen in bacteria.
a.) Are the processes of transduction and conjugation best described as sexual or asexual
processes? Describe the reasoning behind your answer.

They are sexual. Sexual processes create a new genetic combination that was not present in
the previous cells. Both of these processes will typically create new genetic combinations in
the cells that receive the DNA.

b.) Which process, transduction or conjugation, is more likely to result in something favorable
for the bacterium? Describe a major reason in support of your answer.

Conjugation. The genetic material transmitted during conjugation comes from a cell that
survived well enough to manage to engage in this process. The genetic material being sent to
the recipient cell might be helpful to that new cell to survive in similar conditions.
Transduction involves transmitting genetic material from a cell that died due to a
bacteriophage infection, not a great trait to share with other members of your species.

c.) Other than the use of DNA restriction and ligation, what is another feature of transduction
and conjugation that is similar to crossing over that is so often seen during meiosis in eukaryotic
cells?

Both often involve the hybridization of homologous sections of DNA.

d.) Is the process of transduction most likely to be beneficial to an individual bacterial cell or to
the species as a whole? Support your answer by pointing out a specific benefit at the level you
select.

A cell that is living well receives some new DNA by transduction. The most likely outcome
for the cell is that its adaptive state will be disrupted. So transduction is most likely to be
bad for the recipient cell.

There will be a few rare instances in which transduction may make new varieties of
phenotypes possible, and this might be of benefit to the species as it will then have individual
members who might be able to survive selection.

3.) (6 pts) (Lecture 15. See pgs. 408-409.)

The approach to DNA sequencing given in fig. 20.12 shows the use of four distinct types of fluorescent tags so that each labeled ddNTP has a distinct color. When Sanger invented this method he did not use distinctly colored tags. Instead he ran the system with just one of each type of dideoxynucleotide at a time in it, meaning he had to run the system four times to fully sequence a template strand. Assume that the template strand shown in figure 20.12 was sequenced using Sanger's original method and using all the other materials shown in the figure. Fill in the table below to show the relative locations of the resulting bands of DNA in each of the appropriate lanes of a hypothetical electrophoretic gel.

Lanes of Electrophoretic Gel

(Wells of the gel)	Lane using tagged ddATP	Lane using tagged ddCTP	Lane using tagged ddTTP	Lane using tagged ddGTP
(Top of gel)	▮			▮
		▮		
			▮	
				▮
	▮			
	▮			
				▮
(Bottom of gel)		▮		

What would likely happen to the bands shown in the lane using tagged ddATP if the concentration of this dideoxynucleotide was a hundred fold higher than it should be when running the sequencing reaction?

This will make it more likely that the first time that dATP is to be used that it is this tagged ddATP that is used. This will make most of the strands short, and the longer strands will be less likely to form. Thus this lane may have just the one smallest band, and it might lack, or have just very faint, upper bands.

4.) (10 pts) (Lecture 24. See pgs. 396-400.)
See fig. 20.4 in the text and answer the following questions about items shown in it.
a.) When the bacterial plasmid is cut by the restriction enzyme how many linear DNA pieces are created? How many "sticky ends" are present in each of these pieces?

 DNA pieces: *One*   Number of Sticky ends: *Two*

b.) In addition to the AmpR and lacZ genes, what other specific section of DNA would be very useful to have in this bacterial plasmid, and what is its role?

 An origin of replication can be useful so that once a plasmid gets into a bacterium it can be replicated and new copies of it can be made.

c.) Assuming, as shown in fig. 20.4, that the pieces from the bacterial plasmid are mixed with copies of the pieces of linear hummingbird DNA with similar sticky ends and ligated to form a new recombinant plasmid. Is it possible for such a recombinant plasmid, when in bacteria of a colony, to result in a phenotype of antibiotic resistance and a blue color when grown on the culture media used in step 5 of this figure? If not possible, explain why. If possible, diagram a plasmid that would be consistent with this outcome indicating the locations of the genes (or start and ending parts of the genes) lacZ and Amp^R, and humming bird DNA pieces in it.

It could be done if two pieces from the plasmid met up with the start of lacZ matched to the end of lacZ so that this gene is functional. Then the other start and end of lacZ would have a section of bird DNA between them. This produces a recombinant plasmid that contains the bird DNA, but it would have one functional version of the lacZ gene, and two copies of functional AmpR genes. Thus colonies with this plasmid would be antibiotic resistant, and blue in color.
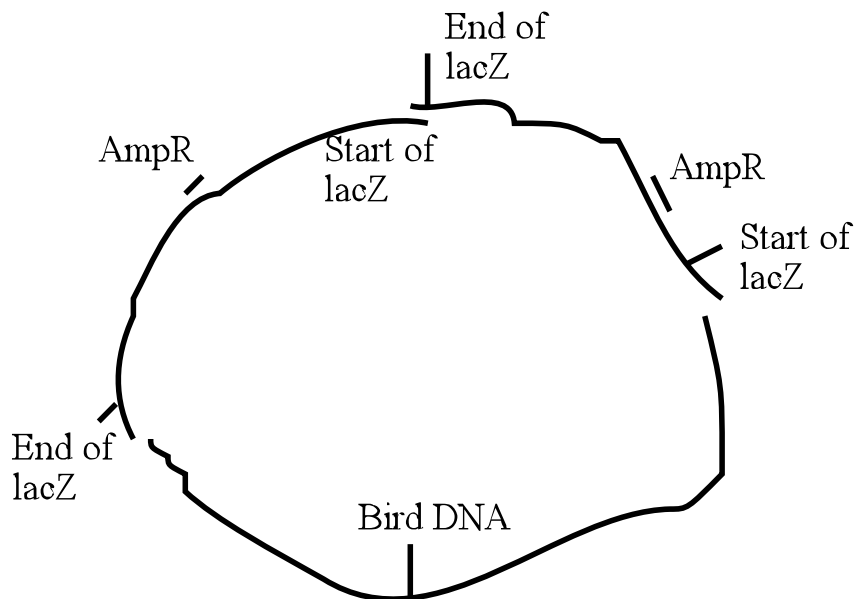
d.) Given just the pieces from the bacterial plasmid (i.e. ignore any hummingbird DNA fragments) could a plasmid be made that would give a bacterial colony antibiotic resistance and have a white color when put on the culture media used in step 5 of this figure? If not possible, explain why. If possible, diagram a plasmid that would be consistent with this outcome indicating the locations of the genes (or start and ending parts of the genes) lacZ and Amp^R in the final plasmid.

It could be done. If two pieces came together with the start ends of two lacZ genes fusing and two ending ends of lacZ genes fusing. This would disable the lacZ, so the colonies would be white.
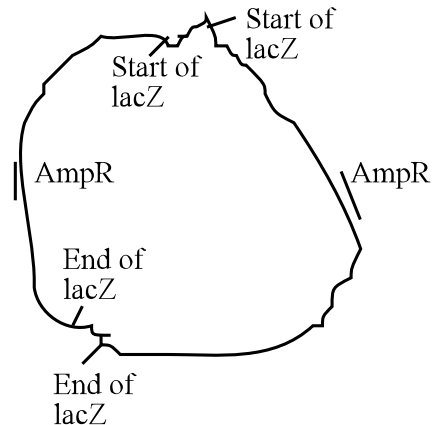
5.) (6 pts) (Lecture 23. See pgs. 344-346, 356-363, 373-377.)

A change in nucleotide sequence in the coding region of a gene might cause a proto-oncogene to be converted into an oncogene, but what are three ways that heritable changes outside of a protein-coding region can also cause the activation of oncogenic activity? Describe for each change that you identify what it might do that could result ultimately in the induction of cancer.

A change in an intron region of the gene could alter the way that splicing of its RNA would be done. This could alter the final mRNA, and so the final protein. If that final protein then is changed in its ability to have its activity regulated due to its change in shape, then it might be always off, and so unable to inhibit the induction of cancer.

A mutation in the promoter region of a gene could alter the ability of a transcription factor complex to be assembled there. This could lower, or raise, the rate of transcriptional initiation. A change in gene expression like this could alter the final production rate of a protein coded for by this gene. With more of the protein made, it would occur out of normal proportion to other proteins. This imbalance might lead to cancer if this protein inhibits tumor suppressor genes, or DNA repair systems.

A change in a distal control element of DNA, such as an enhancer region, could result in a change in the rate of the formation of a transcription factor complex for expression of a gene. This change in rate of gene expression could alter the amount of the protein made, and so might lead to a change in the chance of a cell becoming cancerous.

5

6.) (9 pts) (Lecture 19. See chapter 43 of our text.)
Identify three types of receptors that occur in the mammalian immune system each of which detects non-self items. For each class of receptor that you note, indicate a specific cell type it is located in, and describe a major role it that cell type then plays in the action of immune system.

Toll-like receptors (TLR) occur in the cell membrane of macrophages. When activated these TLRs will initiate a transduction system that will result in these macrophages being more active in their phagocytosis, and in their secreting more chemical signals to influence the activities of other cells.

T cell receptors (TCR) occur in the cell membrane of helpter T cells. When they bind their antigen, as displayed on an MHCII of antigen presenter cells, then they secrete items that increase the activity of other immune system cells. These items can act on the they they are binding with, or on more distal cells.

B cell receptors (BCR) occur in the cell membrane of memory B cells. When they bind their antigen then the memory B cell is induced to undergo cell divisions, and to create more memory B cells and plasma B cells which will secrete antibodies for this type of antigen.

7.) (11 pts) (Lecture 16. See fig. 46.12 and pgs. 1021-1025.)

See the figure at the right in answering the following questions concerning animal gamete formation and fertilization.

a.) Which letter(s) indicate stages of:

    Dikaryotic cell(s):   _D_
    Diploid cell(s):   _A,B_
    Haploid cell(s):   _C_

b.) Label with a line extending from the letter "P" the polar bodies in this figure.

c.) In addition to its nucleus, most animal sperm

    also contribute a _centriole_ to the resulting zygote.

d.) In mammals, what specific structure typically contains the primary oocyte?

    the ovary

e.) Identify and describe the event that would normally occur next after stage "D" of this figure.

        Next would be karyogamy to finish fertilization.

f.) What two receptors, located where and detecting what, are typically involved in many animals' fertilization to ensure species specificity?

 The sperm has a receptor in its cell membrane that detects a species specific protein in the extracellular matrix of the secondary oocyte.
 The secondary oocyte has a receptor in its cell membrane that detect a species specific item displayed in the cell membrane of the sperm.
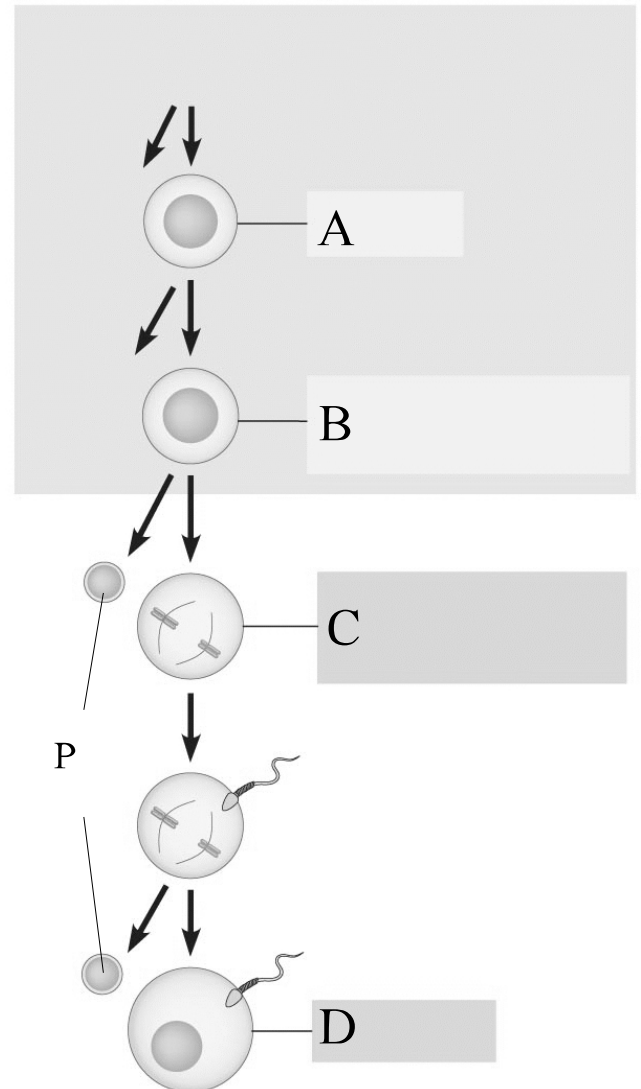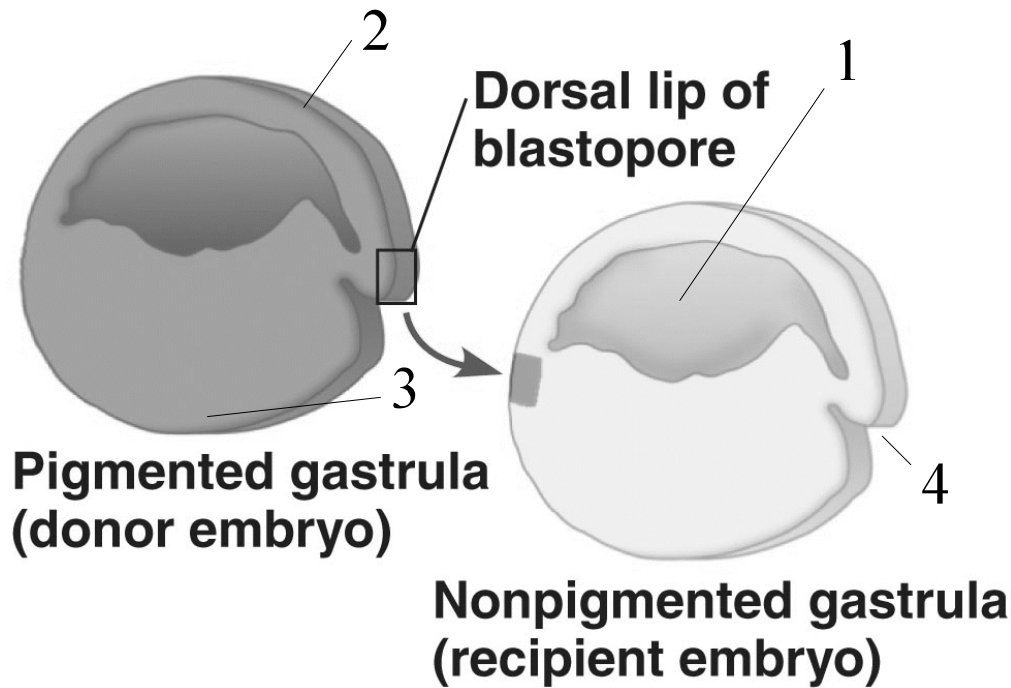
A

B

C

P

D

2

Dorsal lip of blastopore

1

3

Pigmented gastrula (donor embryo)

4

Nonpigmented gastrula (recipient embryo)

8.) (10 pts) (Lecture 20. See pgs. 1025-1042.)

Refer to the above images in answering the following questions.

a.) The above embryos are most likely from which type of animal? (Circle your choice.)

    bird   ***amphibian***   **mammalian**

       Identify one feature visible in the above embryos that supports your answer, and describe how it does so.

This early gastrula is round, like those of frogs. The gastrula of birds and mammals are flattened discs.

b.) Label an example of each of the following in the above embryos by drawing a line to it from the appropriate number.

      1.) Blastocoel.   2.) Area likely to become nervous tissue.

      3.) Vegetal pole.   4.) Location of the future anus.

c.) Fill in the following blanks with the most appropriate term or phrase.

      The dark area transplanted to the pale gastrula will likely secrete items that inhibit

     BMP4 .   This will induce the process of   *gastrulation*   on that side of the recipient embryo, and this may result in the formation of a second

     digestive   organ system.

8

9.) (10 pts) (Lecture 21. See pgs. 223-225, 366-373)
Consider the following questions concerning determination and differentiation in development.
a.) What is one example of a so-called "master" regulatory gene?

<div align="center">*myo-D*</div>

The above gene codes for a product that most likely must do what?

It binds at the promoter site of other genes, most likely genes that code for transcription factors.

If the product coded for by this gene was properly injected into a mature neuron, would this adult cell most likely take on a new phenotype or not? Explain the reasoning behind your answer.

No. During determination the chromatin condensation is altered, and the areas with genes that the myoD protein would attempt to bind at may no longer be accessible to it.

b.) During the development of the frog secondary oocyte it receives *Vgl* RNA from the surrounding cells in the frog follicle. This *Vgl* RNA becomes localized at the vegetal pole as the oocyte matures. A recessive form of the gene for this RNA, *vgl*, has a single nucleotide change, but the RNA it codes for is unable to support subsequent normal embryo pattern formation. Assume that two frogs who are heterozygous for this gene (i.e. both are *Vgl vgl*) mate. What percentage of their tadpoles are likely to have abnormal embryonic development?

0 % (None)

What are two other items that are often sent by nurse/follicle cells to the developing oocyte of many animals?

Lipids

Proteins

c.) If in an animal cell the intracellular proteins Ced-4 and Ced-3 become activated, what is a likely final phenotype of this cell?

<div align="center">*Death of the cell.*</div>

Identify an example in a specific normal developmental pattern that uses this system.

The cell that is in the pore of a developing nematode vulva must be killed so that this pore is opened. This will be done by activiting apoptosis in that cell, via activation of these proteins.

10.) (8 pts) (Lecture 22. See chapter 26 and pg. 699.)
The following is a table of characteristics seen in several chordate species.

	Lizard	Lamprey	Salmon	Lancelet	Rabbit
Notochord	Yes	Yes	Yes	Yes	Yes
Vertebrae	Yes	Yes	Yes	No	Yes
Mammary Glands	No	No	No	No	Yes
Hinged Jaw	Yes	No	Yes	No	Yes
Four Limbs	Yes	No	No	No	Yes

a.) Based on the information in this table, which species would best serve as the out-group?

   Lancelet

b.) By what name are the species in the in-group typically called as a group?

   Vertebrates

c.) Draw the most parsimonious cladogram possible based on the above data. In it, indicate the species, and the point of likely appearance of each characteristic.
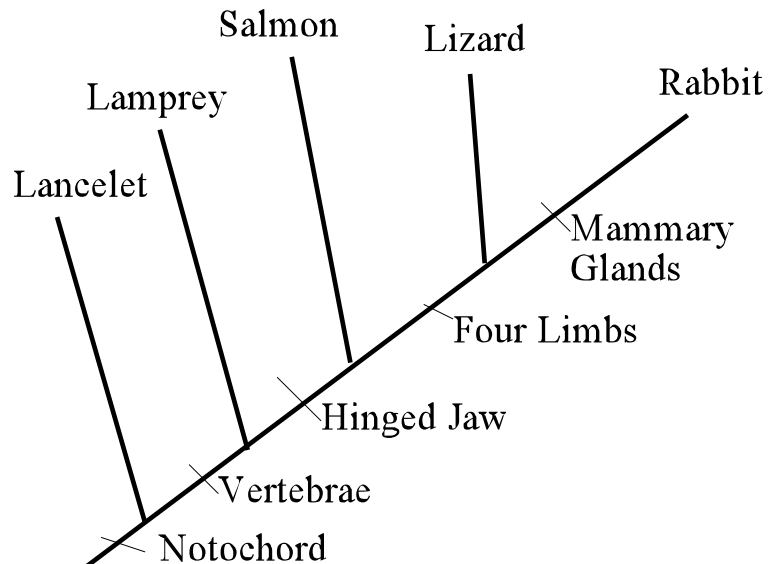
d.) Identify two species from the table that if included with their common ancestor would make up a monophyletic group.

   Lizards and Rabbits.

e.) Identify two species from the table that if included with their common ancestor would make up a paraphyletic group.

   Lamprey and Salmon.

11.) (11 pts) (Lecture 17. See pgs. 625-628, and 801-811.)
Examine the figure shown at the right and answer the following questions about it.

a.) Label an example of each of the following items by drawing a line to it with the appropriate number.

  1.) An ovule.
  2.) A triploid (3N) region.
  3.) An integument.
  4.) A structure, as shown in the figure, capable of creating spores of some type without having to do a lot of growth first.
  5.) The wall of the ovary.

b.) The young plant shown to be growing in the middle of this figure is of which generation?

  young sporophyte
  It gets its food for growth from what immediate source?

  the endosperm

c.) When mature the structure that will surround and include the mature ovule will best be called a:

  fruit
Describe a major function of this structure in the life cycle of flowering plants.

  The fruit plays a large role in the dispersal of the seeds in it. The fruit wall, modified from the ovarian wall, is what is most important in this process.
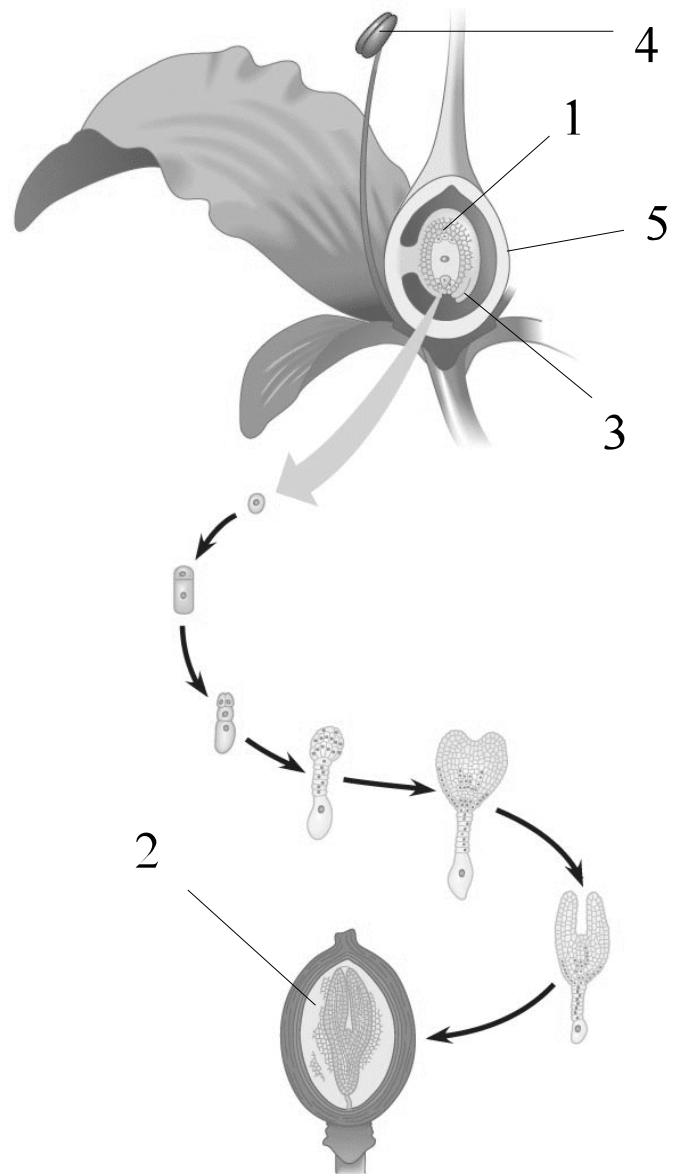
12.) (9 pts) (Lecture 18. See pgs. 610-615.)
In the figure below indicate a location of an example of each of the following items by drawing a line to the item and labeling that line with the appropriate letter.

    a.) Sporophyte   b.) Gametophyte   c.) Sporangium
    d.) Archegonium   e.) Antheridium   f.) Zygote
    g.) Spore
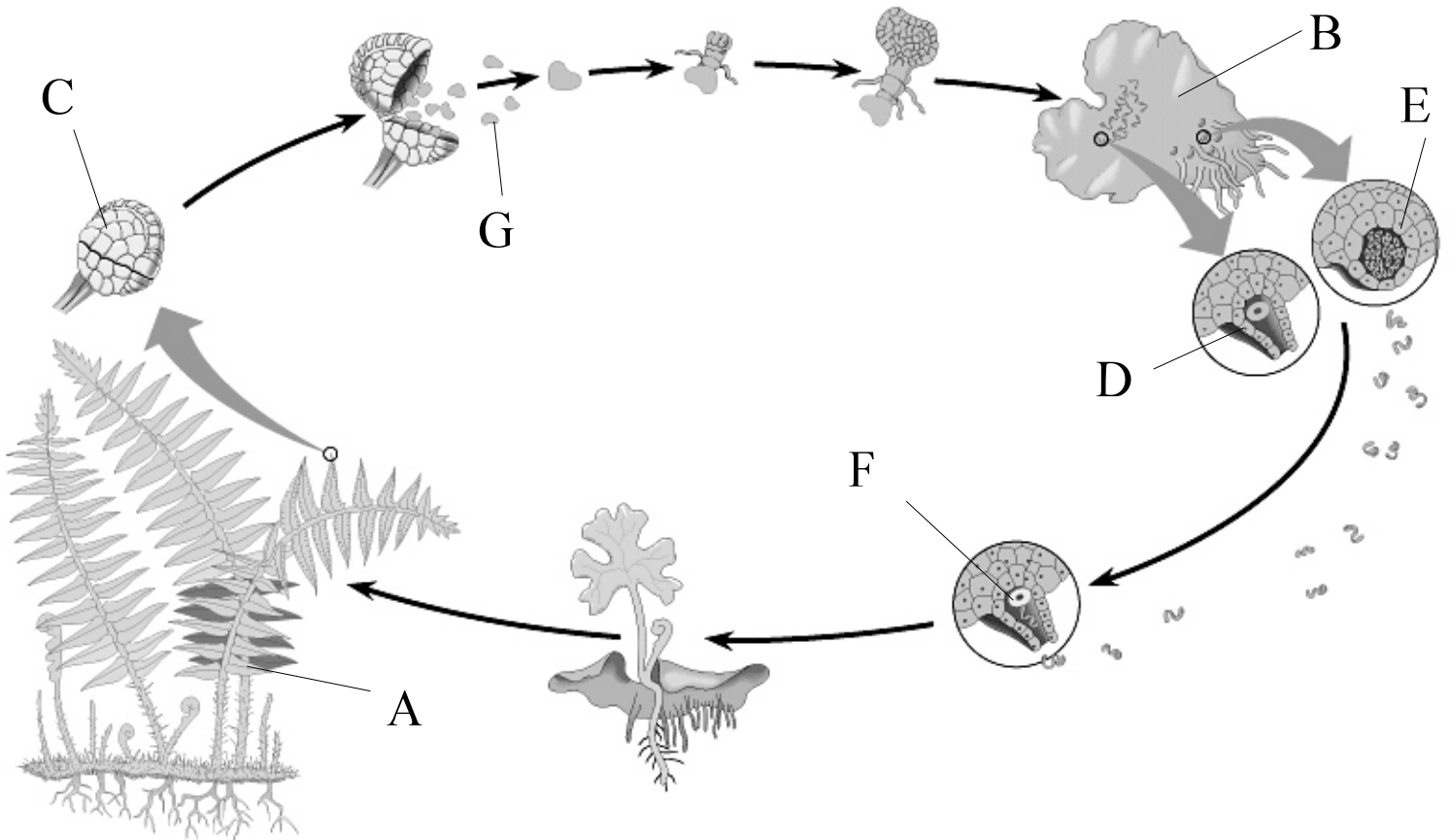
Identify the structure(s) involved in sexual reproduction in the above figure, and note what each does that is sexual.

The sporangia do a sexual act by having cells in it that carry out meiosis and create spores with new genetic combinations.
The sperm, from the antheridia, and the eggs, in the archegonia, engage in the sexual act of fertilization and so create a new genetic combination in a zygote.

BIO 108   2010
Homework set #3   Name: _____
Due 9:00 am, Tuesday, August 3rd. (Text references are indicated for each question.)
        Points earned: _____ = _____ for course.
                                    5
_____
1.) (10 pts.) (Lecture 25. See chapter 22.)
For each of the following issues contrast the common view of scientists concerning it before the
1800s with the common view of scientists about it today.

	Before the 1800s:	Today:
What did they think that the age of the earth was?		
Were species seen as fixed entities or were they thought to be able to vary?		
Were extinctions seen to possible or not?		
Was the environment seen as stable over the long term, or not?		
New species were seen as originating from what?		

2.) (9 pts) (Lecture 26. See chapters 32 and 33.) For each pair of animal groups identify a
specific species as an example of each group, and one distinguishing trait that separates the two
groups in each pair.

Pair:	**Annelida**	**Platyhelminthes**
Members:		
Trait:		

Pair:	**Mollusca**	**Arthropoda**
Members:		
Trait:		

Pair:	**Protostomes**	**Deuterostomes**
Members:		
Trait:		

3.) (6 pts) (Lecture 27. See the articles by Runyon et al. 2006, and Lacroix et al. 2005)
In both the articles by Runyon et al. (2006) and Lacroix et al. (2005) volatile molecules were shown to be able to induce responses. Answer the following questions about aspects of these studies and their findings.

a.) In what way was the mosquitoes behavior altered by the volatile signal it detected? And was this response adaptive for the mosquito (i.e. does the behavior benefit the mosquito)?

b.) What is one way that the plant dodder responds when it detected a volatile signal? Describe how this response may or may not be adaptive for this plant.

c.) Are the bioassays done in these studies quantitative or qualitative bioassays? Explain the reasoning behind your answer.

4.) (7 pts) (Lecture 28. See chapter 24.)
Describe what would be expected to be seen in the fossil record if speciation was faithfully documented in it for each of the following mechanisms, and note in your description how each case would differ from the other.

Gradualistic allopatric speciation:

Speciation by allopolypoidy:

Which type of speciation is most consistent with what was proposed by Darwin?

_____

5.) (12 pts) (Lecture 29. See chapter 23, and figure 14.15.)

The genotypes of a group of people were determined for the alleles of a gene that is involved in the growth of attached versus free earlobes:

    FF (free earlobe)   1445
    Ff (free earlobe)   510
    ff   (attached earlobe)   45

Answer the following questions concerning this population.

a.) What are the allele frequencies? (Show your calculations.)

f(f) =

f(F) =

b.) Given the above allele frequencies, according to the Hardy-Weinberg model, what would be the expected numbers of individuals in the next generation with free earlobes versus attached earlobes out of a total population of 2000 humans? (Show your calculations, and as needed round your final answers off to the nearest whole individual to give the appropriate total.)

Number with free earlobes =

Number with attached earlobes =

c.) Compare the answers you obtained in part (b) to the initial population described in the premise. What does this allow you to conclude about this population?

6.) (6 pts) (Lecture 30. See pgs. 630-632, 804-805.)
For each of the following describe specifically what that process achieves, and what structure(s) of a flowering plant species is(are) typically involved.

Pollination.
Double Fertilization.
Dispersal.

7.) (7 pts) (Lecture 31. See pgs. 507-510, 514-516.)
a.) Assuming that a solution with a lot of organic molecules occurs today, what is likely to happen to these molecules now that would not have happened to them four billion years ago?

b.) Assuming that a new lineage of life arose today by spontaneous generation, what is most likely to happen to these new cells today that would not have likely happened to them four billion years ago?

c.) Assuming that the "RNA world" model is correct, then RNA would have been an early polymer found in life. What are two other polymers that would have also have been present early in life, and which one was likely to have occurred earlier than the other?

d.) What class of reaction typically results in the conversion of monomers into polymers? Describe how a set of conditions that would have likely have occurred on the early earth may have promoted polymer formation.

8.) (10 pts) (Lecture 32. See pgs. 1154-1171.)
The figure below shows South America with the shaded regions indicating distinct biomes.
What biome is indicated by each letter?
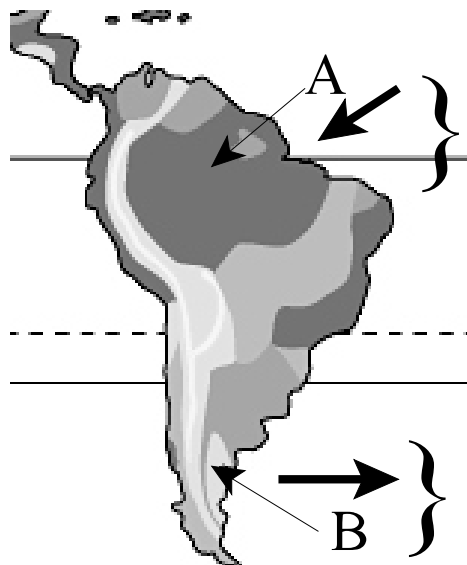
A.) _____

B.) _____

Draw an arrow next to each bracket ("}") indicating the direction of the prevailing winds in the
latitudes each covers.

For each area (A and B) identify two abiotic factors that contribute to the presence of the biome
in that region, and explain how it contributes in each case.

A.)

B.)

9.) (8 pts) (Lecture 33. See pgs. 1181-1186.)

In 2009 the population of humans on Earth was roughly 6.9 billion people. Assume that the average annual birth rate is 15.1 births per thousand people, and the average annual death rate is 7.9 deaths per thousand people, and that the global carrying capacity for humans is nine billion people. Estimate the global population size for each of the subsequent years using the exponential growth equation and the logistic growth equation. (In your calculations round each answer for each year down to the nearest full person for that year before calculating the next...)

New population calculated using:

Year	Exponential growth equation:	Logistic growth equation:
2009	6,900,000,000	6,900,000,000
2010		
2011		
2012		
2013		
2014		
2015		
2016		

The two estimated populations for 2016 differ greatly. In the real world what is a reason for one estimate to be so much lower than the other (i.e., what is happening that accounts for all these missing people)?

BIO 108   2010

Proposed Answers for Homework set #3.

Below are some possible answers to the questions in this homework set. Please note that other answers might receive full or partial credit.

Please look over the proposed answers both so that you can gain a sense of where I was headed with each question, and to give yourself some feedback on the issues raised by these questions.

Anyone who wishes more feedback may come see me about items in this homework set. Please see the course syllabus for information about how to request regrading of any lecture item.

BIO 108   2010
Homework set #3   Name: _____
Due 9:00 am, Tuesday, August 3rd. (Text references are indicated for each question.)
        Points earned: _____ = _____ for course.
                                    5
_____

1.) (10 pts.) (Lecture 25. See chapter 22.)
For each of the following issues contrast the common view of scientists concerning it before the 1800s with the common view of scientists about it today.

	<u>Before the 1800s:</u>	<u>Today:</u>
What did they think that the age of the earth was?	*About 10000 years.*	*About 4.5 billion years.*
Were species seen as fixed entities or were they thought to be able to vary?	*Fixed.*	*Able to vary.*
Were extinctions seen to possible or not?	*No extinctions occur.*	*Extinctions happen.*
Was the environment seen as stable over the long term, or not?	*Relatively stable.*	*Changes over time.*
New species were seen as originating from what?	*From non-living matter.*	*From previous species.*

2.) (9 pts) (Lecture 26. See chapters 32 and 33.) For each pair of animal groups identify a specific species as an example of each group, and one distinguishing trait that separates the two groups in each pair.

Pair:	**Annelida**	**Platyhelminthes**
Members:	*Polychaete (Hesiolyra bergi)*	*Blood flukes (Schistosoma monsi)*
Trait:	*Annelids have body cavities (i.e. coeloms), while platyhelminths lack body cavities.*	

Pair:	**Mollusca**	**Arthropoda**
Members:	*Snails (Gastropoda)*	*Horseshoe crabs (Limulus polyphemus)*
Trait:	*Arthropods periodically molt their exoskeleton, molluscs do not molt.*	

Pair:	**Protostomes**	**Deuterostomes**
Members:	*Crabs (decapods)*	*Humans (Homo sapiens sapiens)*
Trait:	*Protostomes have early determinate cleavage, while deuterstomes do not have determination until much later in development.*	

3.) (6 pts) (Lecture 27. See the articles by Runyon et al. 2006, and Lacroix et al. 2005)
In both the articles by Runyon et al. (2006) and Lacroix et al. (2005) volatile molecules were shown to be able to induce responses. Answer the following questions about aspects of these studies and their findings.
a.) In what way was the mosquitoes behavior altered by the volatile signal it detected? And was this response adaptive for the mosquito (i.e. does the behavior benefit the mosquito)?

The mosquitoes were attracted to people who had malarial parasites in them at a stage of development where they were ready to enter mosquitoes. This response was either neutral or costly to the mosquitoes as it causes the malarial parasite to enter the insect's body and live in them for a while. The cost to the mosquito would depend on how much food these parasites take from their host.

b.) What is one way that the plant dodder responds when it detected a volatile signal? Describe how this response may or may not be adaptive for this plant.

Some odors caused the dodder plants to grow towards higher concentrations of the signal. This is beneficial to these dodder plants as it gets them closer to the plants that release these odors, and these tend to be the plants on which dodder wishes to feed.

c.) Are the bioassays done in these studies quantitative or qualitative bioassays? Explain the reasoning behind your answer.

These are qualitative bioassays. While they detected the presence of certain items by the responses of the organisms, the responses were not correlated in amplitude relative to the amplitude of the signal detected as would be expected in a quantitative bioassay.

4.) (7 pts) (Lecture 28. See chapter 24.)
Describe what would be expected to be seen in the fossil record if speciation was faithfully documented in it for each of the following mechanisms, and note in your description how each case would differ from the other.

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In the fossil record there would be many intermediate forms of the species evident. These different intermediate forms would occur in different areas, each area being where each different species evolved. These forms would shift slowly over time, but could be assembled in sequence so that the original single ancestral species could be identified.

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Any fossils showing the new species would occur suddenly in one location. There would be no intermediate forms evident in the fossil records. Depending on which two species contributed genetic material to the new species it may or may not be possible to determine the two ancestral species, because the new species may be very different in form from either of these two species.

Which type of speciation is most consistent with what was proposed by Darwin?

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$$(510 + 90)/(4000) = (600)/(4000) = 0.15$$

  f(F) =

$$(2890 + 510)/(4000) = (3400)/(4000) = 0.85$$

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  Number with free earlobes =

$$((0.85)^2 + (2)(0.85)(0.15))(2000) = 1955$$

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$$(0.15)^2(2000) = 45$$

c.) Compare the answers you obtained in part (b) to the initial population described in the premise. What does this allow you conclude about this population?

The expected numbers are the same as the observed numbers for each phenotype. This implies that the population that was observed was in Hardy-Weinberg equilibrium. This means that it was relatively large in population size. There was no net selection, or mutation occurring. And any changes due to immigration was balanced by emmigration.

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This achieves the formation of a zygote and a triploid endosperm cell. To do this two sperm, delivered from a pollen tube via a synergid, had to fuse with an egg and a dikaryotic central cell in the female gametophyte in the ovule.

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When done properly this delivers a seed, either in a fruit or not, to a new location. This may be a place where the species has been before, but it may be a location that is entirely new for the species. Structures on the seed, or on the fruit coat, could assist in the dispersal of the seed.

7.) (7 pts) (Lecture 31. See pgs. 507-510, 514-516.)
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d.) What class of reaction typically results in the conversion of monomers into polymers? Describe how a set of conditions that would have likely have occurred on the early earth may have promoted polymer formation.

Condensation reactions typically are used to convert monomers into polymers. These reactions involve the formation of water. Thus anything that removes this water would tend to pull the polymerization forward. This could be done by the drying out of a pond under a hot sun. The residue in the drying mud would tend to have more polymers in it.

8.) (10 pts) (Lecture 32. See pgs. 1154-1171.)
The figure below shows South America with the shaded regions indicating distinct biomes. What biome is indicated by each letter?

A.)   _Tropical rainforest._

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Draw an arrow next to each bracket ("}") indicating the direction of the prevailing winds in the latitudes each covers.

For each area (A and B) identify two abiotic factors that contribute to the presence of the biome in that region, and explain how it contributes in each case.

A.)

Being at the equator the light intensity is high and fairly steady over the year. This keeps the area evenly warm over the year. And this warm air constantly rises and forms clouds producing high amouts of rain throughout the year. Thus warmth and rainfall are two abiotic factors that help determine this biome's location.

B.)

West of this area are the Andes mountains. Given the prevailing winds, this produces a rainshadow effect east of these moutains. Also this area is near to a latitude where air in the upper atmosphere tends to descend, and so there is dry air delivered to the ground. Thus the mountain range and the latitude are two abiotic factors that help to determine this biome's location.

9.) (8 pts) (Lecture 33. See pgs. 1181-1186.)

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| Year | New population calculated using: | |
	Exponential growth equation:	Logistic growth equation:
2009	6,900,000,000	6,900,000,000
2010	6,949,680,000	6,911,592,000
2011	6,999,717,646	6,923,139,379
2012	7,050,115,663	6,934,642,095
2013	7,100,876,495	6,946,100,109
2014	7,152,002,805	6,957,513,384
2015	7,203,497,225	6,968,881,886
2016	7,255,362,405	6,980,205,583

The two estimated populations for 2016 differ greatly. In the real world what is a reason for one estimate to be so much lower than the other (i.e., what is happening that accounts for all these missing people)?

In the logistic model, as the population approaches the carrying capacity there are density-dependent factors that tend to limit the population growth. These factors include such items as a higher rate of disease, and more deaths due to starvation. Thus perhaps the missing people all have died due to limits imposed by the limited carrying capacity.

BIO 108   2010
Homework set #3   Name: _____
Due 9:00 am, Tuesday, August 3rd. (Text references are indicated for each question.)
        Points earned: _____ = _____ for course.
                              5
_____
1.) (10 pts.) (Lecture 25. See chapter 22.)
For each of the following issues contrast the common view of scientists concerning it before the 1800s with the common view of scientists about it today.

	Before the 1800s:	Today:
What did they think that the age of the earth was?		
Were species seen as fixed entities or were they thought to be able to vary?		
Were extinctions seen to possible or not?		
Was the environment seen as stable over the long term, or not?		
New species were seen as originating from what?		

2.) (9 pts) (Lecture 26. See chapters 32 and 33.) For each pair of animal groups identify a specific species as an example of each group, and one distinguishing trait that separates the two groups in each pair.

Pair:	**Annelida**	**Platyhelminthes**
Members:		
Trait:		

1

Pair:	**Mollusca**	**Arthropoda**
Members:		
Trait:		

Pair:	**Protostomes**	**Deuterostomes**
Members:		
Trait:		

3.) (6 pts) (Lecture 27. See the articles by Runyon et al. 2006, and Lacroix et al. 2005)
In both the articles by Runyon et al. (2006) and Lacroix et al. (2005) volatile molecules were shown to be able to induce responses. Answer the following questions about aspects of these studies and their findings.
a.) In what way was the mosquitoes behavior altered by the volatile signal it detected? And was this response adaptive for the mosquito (i.e. does the behavior benefit the mosquito)?

b.) What is one way that the plant dodder responds when it detected a volatile signal? Describe how this response may or may not be adaptive for this plant.

c.) Are the bioassays done in these studies quantitative or qualitative bioassays? Explain the reasoning behind your answer.

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Describe what would be expected to be seen in the fossil record if speciation was faithfully documented in it for each of the following mechanisms, and note in your description how each case would differ from the other.

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    FF (free earlobe)   1445
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Answer the following questions concerning this population.
a.) What are the allele frequencies? (Show your calculations.)

f(f) =

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Number with free earlobes =

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c.) Compare the answers you obtained in part (b) to the initial population described in the premise. What does this allow you to conclude about this population?

6.) (6 pts) (Lecture 30. See pgs. 630-632, 804-805.)
For each of the following describe specifically what that process achieves, and what structure(s)
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Pollination.

Double Fertilization.

Dispersal.

7.) (7 pts) (Lecture 31. See pgs. 507-510, 514-516.)
a.) Assuming that a solution with a lot of organic molecules occurs today, what is likely to happen to these molecules now that would not have happened to them four billion years ago?

b.) Assuming that a new lineage of life arose today by spontaneous generation, what is most likely to happen to these new cells today that would not have likely happened to them four billion years ago?

c.) Assuming that the "RNA world" model is correct, then RNA would have been an early polymer found in life. What are two other polymers that would have also have been present early in life, and which one was likely to have occurred earlier than the other?

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What biome is indicated by each letter?

A.) _____

B.) _____

Draw an arrow next to each bracket ("}") indicating the direction of the prevailing winds in the latitudes each covers.

For each area (A and B) identify two abiotic factors that contribute to the presence of the biome in that region, and explain how it contributes in each case.

A.)

B.)

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BIO 108   2010

Proposed Answers for Homework set #3.

Below are some possible answers to the questions in this homework set. Please note that other answers might receive full or partial credit.

Please look over the proposed answers both so that you can gain a sense of where I was headed with each question, and to give yourself some feedback on the issues raised by these questions.

Anyone who wishes more feedback may come see me about items in this homework set. Please see the course syllabus for information about how to request regrading of any lecture item.

BIO 108   2010
Homework set #3   Name: _____
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1.) (10 pts.) (Lecture 25. See chapter 22.)
For each of the following issues contrast the common view of scientists concerning it before the 1800s with the common view of scientists about it today.

	Before the 1800s:	Today:
What did they think that the age of the earth was?	*About 10000 years.*	*About 4.5 billion years.*
Were species seen as fixed entities or were they thought to be able to vary?	*Fixed.*	*Able to vary.*
Were extinctions seen to possible or not?	*No extinctions occur.*	*Extinctions happen.*
Was the environment seen as stable over the long term, or not?	*Relatively stable.*	*Changes over time.*
New species were seen as originating from what?	*From non-living matter.*	*From previous species.*

2.) (9 pts) (Lecture 26. See chapters 32 and 33.) For each pair of animal groups identify a specific species as an example of each group, and one distinguishing trait that separates the two groups in each pair.

Pair:	**Annelida**	**Platyhelminthes**
Members:	*Polychaete (Hesiolyra bergi)*	*Blood flukes (Schistosoma monsi)*
Trait:	*Annelids have body cavities (i.e. coeloms), while platyhelminths lack body cavities.*	

1

Pair:	**Mollusca**	**Arthropoda**
Members:	*Snails (Gastropoda)*	*Horseshoe crabs (Limulus polyphemus)*
Trait:	*Arthropods periodically molt their exoskeleton, molluscs do not molt.*	

Pair:	**Protostomes**	**Deuterostomes**
Members:	*Crabs (decapods)*	*Humans (Homo sapiens sapiens)*
Trait:	*Protostomes have early determinate cleavage, while deuterstomes do not have determination until much later in development.*	

3.) (6 pts) (Lecture 27. See the articles by Runyon et al. 2006, and Lacroix et al. 2005)
In both the articles by Runyon et al. (2006) and Lacroix et al. (2005) volatile molecules were shown to be able to induce responses. Answer the following questions about aspects of these studies and their findings.
a.) In what way was the mosquitoes behavior altered by the volatile signal it detected? And was this response adaptive for the mosquito (i.e. does the behavior benefit the mosquito)?

The mosquitoes were attracted to people who had malarial parasites in them at a stage of development where they were ready to enter mosquitoes. This response was either neutral or costly to the mosquitoes as it causes the malarial parasite to enter the insect's body and live in them for a while. The cost to the mosquito would depend on how much food these parasites take from their host.

b.) What is one way that the plant dodder responds when it detected a volatile signal? Describe how this response may or may not be adaptive for this plant.

Some odors caused the dodder plants to grow towards higher concentrations of the signal. This is beneficial to these dodder plants as it gets them closer to the plants that release these odors, and these tend to be the plants on which dodder wishes to feed.

c.) Are the bioassays done in these studies quantitative or qualitative bioassays? Explain the reasoning behind your answer.

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7

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Tuesday, July 20th   Name: _____
      Points earned: _____ = _____ for course.
                        70
Please keep your answers in the spaces provided. Good luck!

1.) Indicate the location(s) of each of the following items or processes in the figure to the right by drawing a line to specific spot(s) in the figure and labeling each with the appropriate letter. (7 pts)

a.) Diploid stage(s).
b.) Plasmogamy.
c.) Meiosis.
d.) Mitotic division(s).

What is one difference between the life cycle shown here and that seen in a typical animal species?
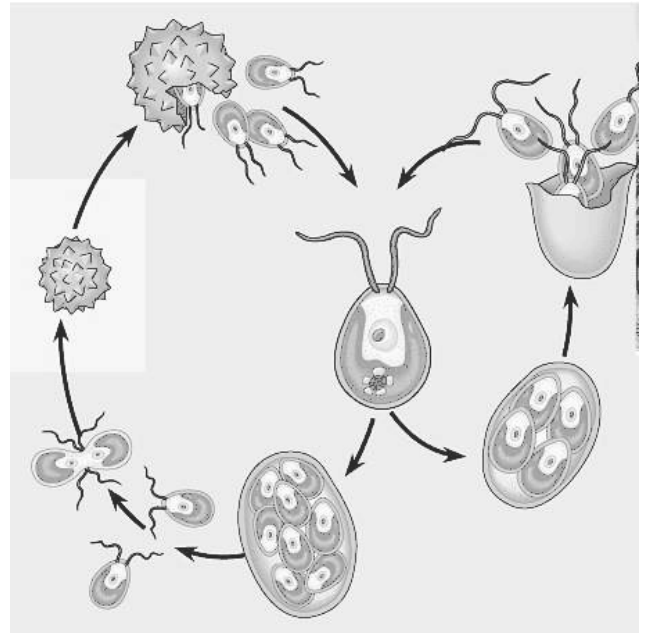
2.) The following statements describe zygotes and spores. Which one statement is INCORRECT? (1 pt)

    a.) Both zygotes and spores are single-celled structures.
    b.) Zygotes and spores are just two of the generations that alternate in the life cycle of
        plants.
    c.) Spores can only go through mitotic cell division, while depending on the type of life
        cycle zygotes may go through either mitotic or meiotic cell division.
    d.) Zygotes are typically not haploid, while spores normally are haploid.
    e.) The production of spores directly requires cell division, while the production of
        zygotes does not directly require any type of cell division.

        Answer: _____

3.) What is one difference between the life cycle of a typical fungus compared to that of a typical plant? (2 pts)

4.) Which is a CORRECT statement concerning the eukaryotic cell cycle? (2 pts)

    a.) Passage through stages of the cell cycle typically involves changes in the concentration of CDK proteins by changes in the rate of their gene expressions.
    b.) In most eukaryotic cells, passage through the cell cycle results in daughter cells with a different ploidy relative to the mother cell.
    c.) For most cells the cell cycle is "arrested" in S-phase for most of its time.
    d.) Typically to pass a check point in the cycle, a CDK binds with a cyclin resulting in the phosphorylation of specific proteins.
    e.) Regulation of the cell cycle involves the use of one type of cyclin and many types of CDK proteins.

    Answer: _____

5.) A species has its genes scattered amongst five types of chromosomes. A diploid cell of this species undergoes the processes of mitosis and cytokinesis to produce two daughter cells. Consider each of the following stages of these processes, assuming everything happens in the normal fashion. (4 pts)

The number of chromosomes present during metaphase: _____
The number of chromatids present during prometaphase: _____
How many homologous pairs of chromosomes are present during metaphase?   _____
The total number of sets of the genome present in the cell during anaphase: _____

6.) There are a number of different things that are accomplished in the process of mitotic cell division in a typical eukaryotic cell. Of the events listed below, which is most likely to occur THIRD in a typical cell that is going through mitosis? (2 pts)

    a.) Kinetochore microtubules bind to a region of the centromeres of the chromosomes.
    b.) Segregation of complete genomic sets of chromosomes occurs.
    c.) The nuclear envelope membranes are converted from flat bilayers into spherical vesicles.
    d.) The number of chromosomes in the cell doubles as double-chromatid chromosomes are split into pairs of single-chromatid chromosomes.
    e.) Vesicles fuse to one another to form a double membrane layer.

    Answer: _____

7.) There are two cell divisions in meiosis and one in mitosis. A **major** benefit of the second division in meiosis is to: (1 pt)

    a.) separate alleles in the sister chromatids that are no longer identical.
    b.) increase the number of cells produced by two-fold.
    c.) reduce the size of the resulting cells made, so that they will be able to grow faster.
    d.) reduce the number of chromosomes by one-half.
    e.) permit independent assortment of homologous pairs of chromosomes.

        Answer: _____

8.) The following cross is done: CCDdAaBBEe X ccDdAabbEe . From this cross what is the probability of producing each of the following genotypes in an offspring? (Assume that the genes are fully unlinked from each other.) (4 pts)

    a.) CcDDAaBbee   _____

    b.) CCDDAABBEE   _____

9.) Compare and contrast the principles of segregation and of independent assortment. (4 pts)

10.) Note the pedigree shown at right. Filled in symbols show a specific trait. Boxes represent males and circles represent females. The simplest model that accounts for the data is that the trait is due to a mutated genetic factor that is an autosomal recessive allele (t) of a single gene (T). (3 pts)

a.) Give the most likely genotypes of individuals:

     A: _____   B: _____

b.) In the pedigree, circle two parents and one of their offspring that supports this trait being due to an autosomal recessive allele, instead of an autosomal dominant or a sex-linked recessive allele.
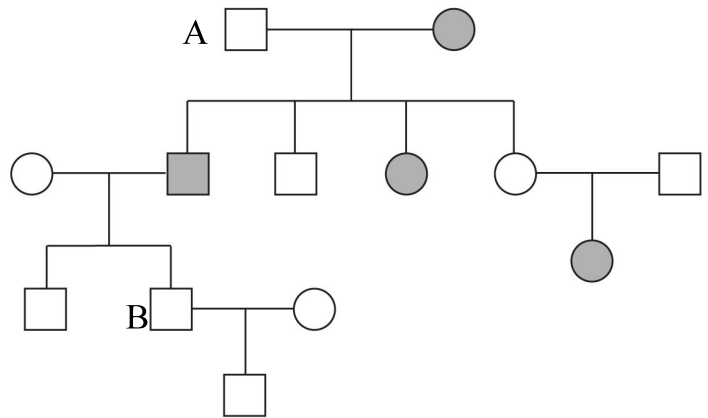
11.) Match the letter of each term with the statement which best describes it. (5 pts)

    a.) Incomplete Dominance   b.) Codominance
    c.) Pleiotropy   d.) Epistasis
    e.) Polyallelic   f.) Polygenic

  _____ The expression of a gene causes changes in more than one phenotypic trait.
  _____ In the heterozygous state the phenotype is intermediate between the
           homozygous dominant and recessive states.
  _____ In a population there is more than one type of allelic form of a gene present.
  _____ In a homozygous state for this gene the expression of other genes can be altered.
  _____ In the heterozygous state the phenotype is entirely new and not blended.
  _____ A phenotype that is influenced by the genotypic
           state of many genes.

12.) A women who does not have the phenotype for hemophilia has a father who has hemophilia (a recessive X sex-linked condition). If she marries a man who also does not have the hemophilia phenotype, then what is the probability that: (4 pts)
    a.) A son of their's will have hemophilia:

          _____

    b.) A daughter of their's will have hemophilia:

          _____
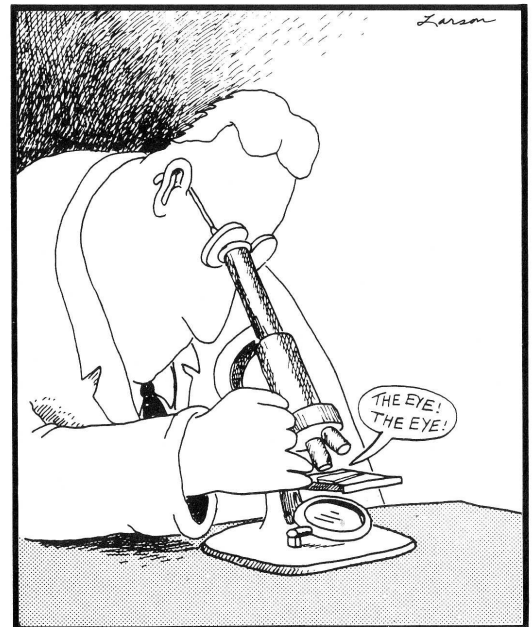
13.) In a test cross of fruit flies a wild type fly (heterozygous for gray body color and for red eyes) is mates with a homozygous recessive fly (black body color and purple eyes). (5 pts)
The offspring were:

965	Wild type.	935	black body and purple eyes.
43	Gray body and purple eyes.	57	Black body and red eyes.

a.) Based on these data, are the genes for body color and eye color most likely genetically linked or unlinked?

_____

b.) Which of the following would be the best way to represent the above cross?

a.) GgRr X ggrr   b.) Gg/Rr X gg/rr

c.) Gg/rr X gg/Rr   d.) GR/gr X gr/gr   e.) Gg/gg X Rr/rr

Answer: _____

c.) Draw the chromosomes that hold these genes that would be present a cell in the wild type fly used in the above cross, when it was in metaphase I of meiosis. Assume no crossing over, but show the position of each of the alleles for each gene.

14.) Which is NOT consistent with the findings of Morgan's initial experiments with white-eyed fruit flies? (1 pt)

a.) That a gene for eye color is located on the X chromosome.
b.) That male fruit flies are hemizygous for most of the genes found on their X chromosome.
c.) That the Y chromosome often lacks versions of genes located on the X chromosome.
d.) That all the genes coding for sex-related traits are located on the sex chromosomes.
e.) The inheritance of a gene for eye color correlates with the inheritance of the sex chromosomes.

Answer: _____

15.) Which is NOT a way that fungi often acquire food? (1 pt)

   a.) They get a plant to secrete organic matter to them.
   b.) Food is acquired by the trapping and killing of soil nematodes.
   c.) Extraorganismal organic matter is degraded by hydrolytic enzymes secreted by the
       fungus supplying the fungus with monomers.
   d.) Organic matter is ingested and digested in intracellular food vacuoles.
   e.) An algae takes a liking to a fungi and forms a lichen in which the fungus is fed food.

       Answer: _____

16.) The following figures represent structures that are typical of various groups of fungi. (3 pts)
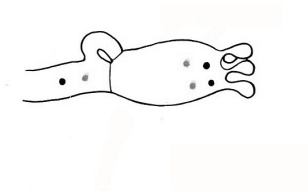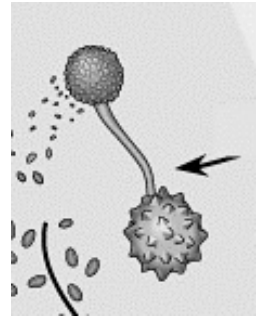
       A.   B.   C.

Which is normally produced by basidiomycetes?   _____
Which is normally produced by zygomycetes?   _____
What is a major and distinctive function of the products made by of these structures?

   _____

17.) To carry out X-ray diffraction analysis of a protein's structure it is most critical to: (1 pt)

   a.) Isolate the gene that codes for that protein.
   b.) Produce homogenous crystals that are made from just that protein.
   c.) Put special isotopes in the amino acids of the protein so that the X-rays are
       diffracted.
   d.) Denature the protein to expose its primary structure.
   e.) Confirm that protease treatments will denature the protein being studied.

       Answer: _____

18.) Which of the following statements concerning aspects of the Hershey-Chase experiment is **false**? (2 pts)

    a.) Sulfur is present in protein, but not DNA; phosphorus is present in DNA, but not often in protein.

    b.) The purpose of the blender was to detach viruses and viral coats from the bacteria.

    c.) Subsequent generations of T2 bacteriophage, grown in the absence of radioisotopes, contained ^{32}P, but very little ^{35}S.

    d.) A conclusion of the Hershey-Chase experiment was that T2 injects the DNA from its head into the bacterium.

    e.) In their experiment most of the ^{32}P-labeled DNA ended up in the supernatant after centrifugation.

    Answer: _____

19.) The molecule shown at right is ATP. Is ATP used as a monomer for RNA or DNA, or both, and why? (1 pt)

    a) Both DNA and RNA. Since adenine is a base found in both of these polymers.

    b.) Just DNA. UTP, with uracil, is used in RNA.

    c.) Neither. ATP is made in the cytoplasm and mitochondria and so is not available for nucleic acid synthesis in the nucleus.

    d.) Just RNA. The sugar in the ATP molecule is a ribose, not a deoxy-ribose, and so will be used to make RNA not DNA.

    e.) Just RNA. To make DNA you need to have nucleic acids in pairs to form the double strand. RNA is normally single-stranded.
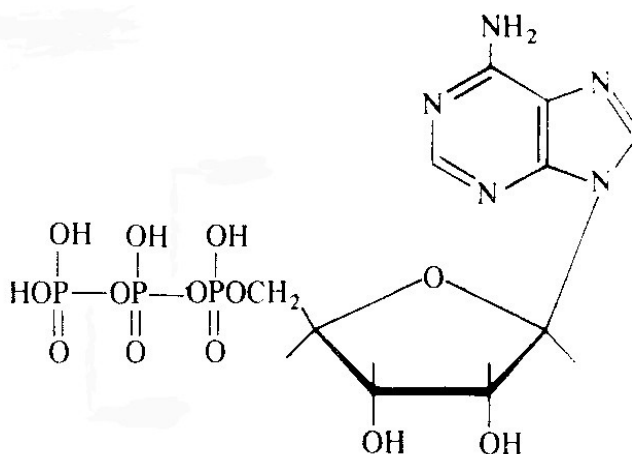
    Answer: _____

20.) What feature of DNA did Chargaff discover that was relevant to its being the carrier of genetic information? (2 pts)

21.) During DNA replication: (6 pts)
a.) Identify the monomers from which new DNA polymers are made.

b.) As each monomer is added what is released?

c.) What enzyme catalyzes the addition of these monomers to the growing DNA polymer?

d.) What determines which type of monomer is next added by the enzyme to an elongating DNA strand?

22.) Before the completion of the replication of DNA, for at least a short time you would expect to find some RNA: (1 pt)

   a.) only near the origins of replication.
   b.) in parts of both the leading and lagging strands.
   c.) just in the leading strands.
   d.) in the part of the DNA that goes on to be translated.
   e.) in just the telomeric regions.

      Answer: _____

23.) Three places in a eukaryotic cell where transcription is often done are: (3 pts)

24.) Rank the following items from first (1) to last (6) as they would occur during typical eukaryotic gene expression. (6 pts)

_____ Spliceosomes interact with a primary transcript.
_____ A transcription factor interacts with DNA.
_____ Activated splicer binds to a mature transcript in the cytosol and cleaves it.
_____ RNA polymerase binds at the promoter region.
_____ A "hairpin loop" of RNA induces RNA polymerase to unbind from DNA.
_____ A mature transcript is exported from a nucleus.

25.) Starting with unactivated tRNA while in the elongation phase of translation, which of the following would then occur next **THIRD** in order? (2 pts)

a.) Binding of a peptide to the amino acid of the amino acyl-tRNA.
b.) The attachment of an amino acid at the 3' end of the tRNA.
c.) Interaction of the codon on the mRNA with the anti-codon on the amino acyl-tRNA.
d.) Movement of the peptidyl-tRNA from the A site to the P site of the ribosome.
e.) The movement of the amino acyl-tRNA into the A site of the ribosome.

Answer: _____

26.) In the genetic code (at right), which amino acid is coded for if the triplet nucleotide sequence 3'-GTC-5' is in the template DNA strand? (2 pts)

a.) Glutamine (Gln)
b.) Leucine (Leu)
c.) Tyrosine (Tyr)
d.) Proline (Pro)
e.) Serine (Ser)

Answer: _____

Second base

First base (5' end)

	U	C	A	G	Third base (3' end)
U	UUU Phe / UUC Phe / UUA Leu / UUG Leu	UCU / UCC / UCA / UCG Ser	UAU Tyr / UAC Tyr / UAA Stop / UAG Stop	UGU Cys / UGC Cys / UGA Stop / UGG Trp	U C A G
C	CUU / CUC / CUA / CUG Leu	CCU / CCC / CCA / CCG Pro	CAU His / CAC His / CAA Gln / CAG Gln	CGU / CGC / CGA / CGG Arg	U C A G
A	AUU Ile / AUC Ile / AUA Ile / AUG Met or start	ACU / ACC / ACA / ACG Thr	AAU Asn / AAC Asn / AAA Lys / AAG Lys	AGU Ser / AGC Ser / AGA Arg / AGG Arg	U C A G
G	GUU / GUC / GUA / GUG Val	GCU / GCC / GCA / GCG Ala	GAU Asp / GAC Asp / GAA Glu / GAG Glu	GGU / GGC / GGA / GGG Gly	U C A G

9

27.) Describe a specific role that each of two (just 2!) of the following items plays in the process of translation. (4 pts)

Release Factors:
Glycine-tRNA Synthetase:
Signal Recognition Particle:
Signal Peptide:

28.) Rank the following steps associated with induction of bacterial gene expression in the order they would occur from first (1) to last (4). (4 pts)

_____ A repressor leaves the promoter region of the gene.
_____ An inducer binds to the repressor protein.
_____ RNA polymerase binds to the promoter region.
_____ Transcription is initiated.
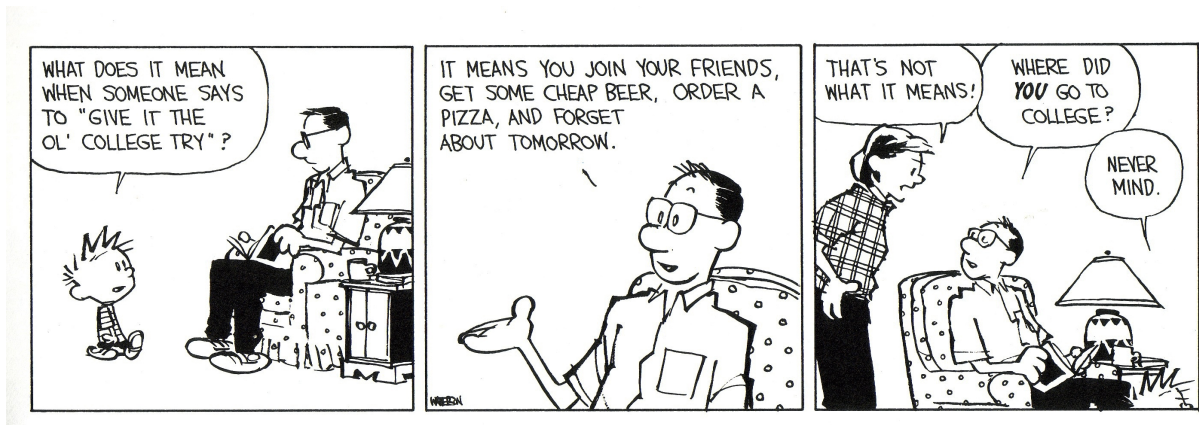
29.) Describe a major role of two (just 2!) of the following in the control of gene expression and state the level of control at which it typically occurs. (4 pts)

Supercoiling:
Riboswitches:
Repressor proteins:

30.) A common covalent modification of DNA that can alter the rate of a gene's expression is

_____ . While a modification done to histones that also can influence

local gene expression is _____ . (2 pts)

11
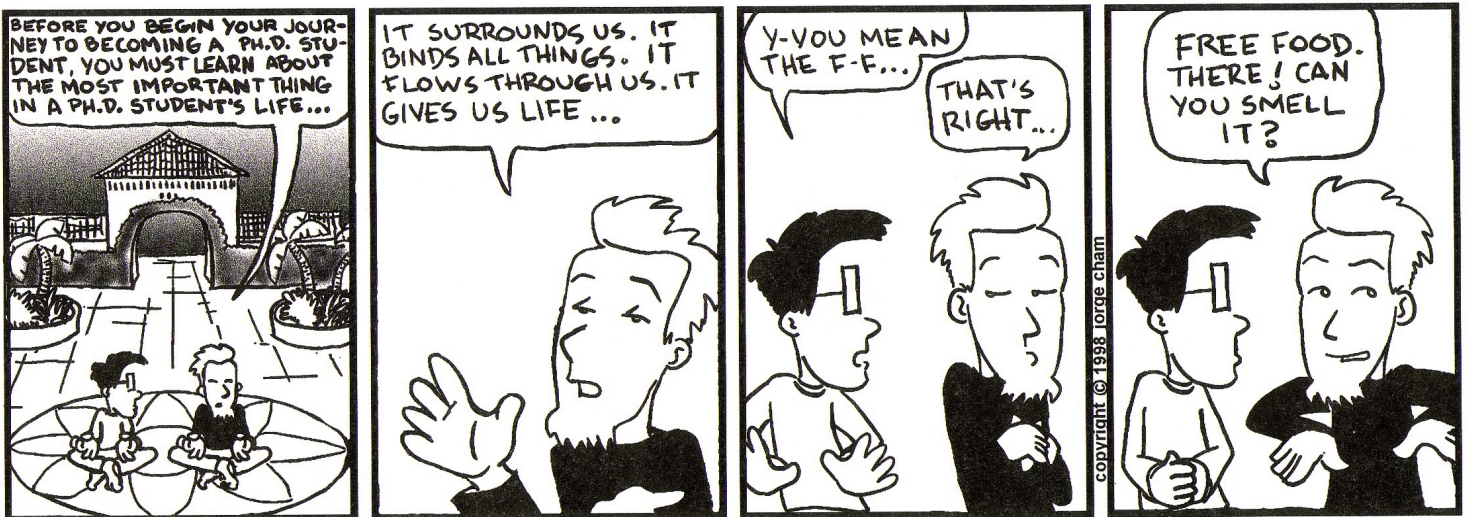
31.) Describe how each of three (just 3!) of the following could have an influence on the total rate of on gene expression in an organism. (I.e. what would it do and how does that alter gene expression rates?) (6 pts)

Production of plasmids:
Transcription factors:
Distal control elements:
Histones:
Dicer enzyme:

Optional Bonus Question. Any points earned in an answer to this question will be substituted for up to five missed points out of the seventy that this exam is worth. (Please limit your answer to this side of this page.)

What are transcription factors and what must they do? Be sure to note how the actions of transcription factors relate to the final phenotype of a cell. In your answer identify what item(s) transcription factors typically interact with, and describe a way that the activity of a transcription factor could be controlled post-translationally. What in the cell determines the combination(s) of transcription factors that are present at any given time?

Exam #1   Proposed Answers:

Below are proposed answers for the questions in this exam.

Be aware that for many of these questions other answers exist that may also be acceptable either for full or partial credit.

1.) (See fig. 28.22, on page 592 of our text.)
    a.) Only the central zygote is diploid.
    b.) The two cells in the lower left.
    c.) Occurs in the upper left.
    d.) The steps going to the lower right and left.

    This life cycle has no multicellular stage,
    as would be seen in an animal life cycle.
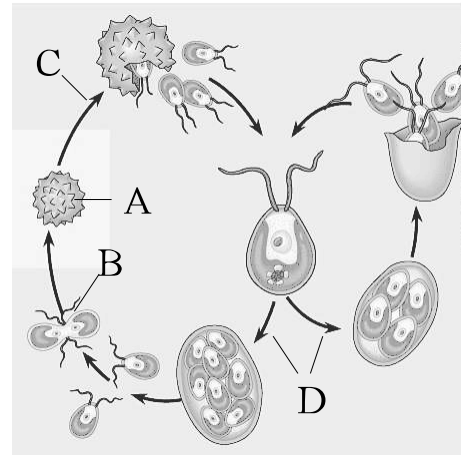
2.) B.

3.) Fungi have a multicellular (N+N) stage that plants do not have.

4.) D.
5.) Down the rows the answers are: 10, 20, 5, and 4.
6.) D.
7.) A.
8.) a.) 1/32   b.) 0 (It can not be done.)

9.)   Segregation calls for keeping sets of the genome together as full sets, either 1N sets or 2N sets. So it ensures a complete set of the genome and does not produce genetic diversity. It occurs in all types of cell divisions.
      Independent assortment normally only occurs during meiosis, and typically produces new genetic combinations. It occurs at a level below that of the full set of the genome, and so can be done without violating the principle of segregation.

10.)   a.) A: Tt   B: Tt
       b.) The wild type male and femae in the second generation, who have a daughter with the trait, would indicate that only an autosomal recessive allele condition is possible.

11.) Down the rows the letters would be: C, A, E, D, B, F.
12.)   a.) 1/2   b.) 0 (it can not happen.)

13.)   a.) linked
        b.) D.
        c.) One homologous pair, with GR on the chromatids of one chromosome and gr on the
             chromatids of the other chromosome.

14.) D.
15.) D.
16.)   A.
        C.
        Dispersal.
17.) B.
18.) E.
19.) D.
20.)   He found that the proportions of the four bases do vary from species to species. This
implies that it is possible for DNA to differ between species, and so could hold information in
itself.
21.)
        a.) dATP, dCTP, dGTP, dTTP.
        b.) Pyrophosphate (PPi).
        c.) DNA polymerase.
        d.) The new nucleotide that is added must have a base that is complementary with the
             next nucleotide in the template strand of DNA.
22.) B.
23.)   The nuclear space of the cell. The matrix space of the mitochondria.
        And the stromal space of the chloroplasts.
24.) The numbers down the rows would be: 4, 1, 6, 2, 3, 5.
25.) C.
26.) A.
27.) (Only two answers were needed here.)
        Release factors: These are proteins that bind at the A site when a stop codon is being
             read. It promotes the release of the polypeptide during termination of translation.
        Glycine-tRNA synthetase: This enzyme will catalyze the covalent bonding of the amino
             acid glycine to its appropriate tRNA. This activation of the tRNA is needed so
             that a supply of AA-tRNAs are available during translation.
        Signal Recognition Particle: A riboprotein particle that recognized the signal peptide of
             a growing polypeptide, and then promotes the binding of the translating
             ribosome to the ER for continuation of translation there.
        Signal Peptide: The first few amino acids of the N-terminal of a growing polypeptide
             that signals for the binding of the SRP.
28.) The numbers down the rows would be: 2, 1, 3, 4.

29.) (Only two answers were needed here.)

      Supercoiling: This is a pre-transcriptional level of control. The twist in the DNA influences its shape, and so influences the binding of other items with it. This can alter the rate of transcriptional initiation.

      Riboswitches: These are in the untranslated regions (UTR) of mRNAs that fold and form binding sites. Binding of its ligand alters the shape of the mRNA, and this can alter the chance of its initiating translation. So this is a pre-translational level of control.

      Repressor proteins: These proteins bind to specific regions of DNA, called operaters, in the promoters of genes or operons. The binding of ligands by this protein may alter its ability to bind to the DNA. When the repressor is bound to the DNA it can block transcriptional initiation, and so this is a type of negative control of gene expression. So this is a ranscriptional level of control.

30.) .... methylation.... acetylation...

31.) (Only three answers were needed here.)

      Production of plasmids: Each plasmid contains new copies of genes from the genome. Increasing the number of copies of the genes increases the rate of gene expression.

      Transcription factors: These proteins bind to the DNA and to the DNA polymerase. When in the right combinations they can increase the rate of transcriptional initiation of a gene.

      Distal control elements: These are regions of DNA often thousands of nucleotides away from a gene. Proteins bind to these elements and then interact with other proteins to influence the formation of transcriptional initiation complexes to initiation gene transcription.

      Histones: These proteins form nucleosomes, about which DNA is wrapped. This condensation of the DNA can be altered to either make transcriptional initiation more or less likely to be started.

      Dicer enzyme: This enzyme cuts up double-stranded RNA to produce small RNAs that are then used in the single-stranded form in RNA interference. They target nucleases to their complement, and this can destroy selected mRNAs and so lower expression of the genes coding for these mRNAs.

Please keep your answers in the spaces provided. Good luck!

1.) A bacterium (*E. coli*) is exposed to a constructed bacteriophage that has a type T3 phage protein coat, but contains two copies of the HIV RNA genome and the enzyme reverse transcriptase. Assuming that the cell has the bacteriophage contents inserted into itself, which is the most likely outcome? (2 pts)

    a.) The cell will be taken over, and will make more HIV viral particles, not bacteriophage
           particles.
    b.) The bacteriophages that end up exiting this infected cell will carry RNA in them based
           on the HIV RNA genome.
    c.) DNA will be made based on the RNA template, but the *E. coli* will be unable to
           express the viral genes present in it properly, so no HIV virons will be made.
    d.) The reverse transcriptase will not work in a prokaryotic cell due to the absence of the
           nucleotide triphosphates needed to make the DNA.
    e.) The HIV genome will be altered by exposure to the T3 phage proteins such that it will
           code for bacteriophage proteins rather than HIV proteins.

        Answer: _____

2.) Select two (just 2!) of the following. For each item you select state: An enzymatic ability typically associated with that class of viruses, state the enzyme's name, and a specific function it carries out. (4 pts)

RNA viruses.
Retroviruses.
Temperate viruses.

3.) Of the following, which choice BEST distinguishes typical members of the Domain Bacteria from all other species? Most bacteria are distinguished by: (2 pts)

    a.) lacking a membrane-bound nucleus and a cell wall, but they have membranes made from lipids that span the membranes.
    b.) lacking membrane-bound organelles, but do have linear chromosomes.
    c.) lacking a cytoskeleton, except for microtubules in their flagella.
    d.) lacking ribosomes, but having a plasma membrane.
    e.) lacking of a membrane-bound nucleus, typically having circular chromosomes, and often they have cell walls containing peptidoglycans.

    Answer: _____

4.) Which is **NOT** an advantage of using plasmids to create new genetic combinations in *E. coli*? (2 pts)

    a.) Several genes can be put together into one plasmid and moved as a group.
    b.) Circular DNA is less likely to be degraded by *E. coli* than linear DNA.
    c.) Plasmids can replicate autonomously of the rest of the DNA in *E. coli*.
    d.) Plasmids do not need to insert into the host DNA in order to function.
    e.) Genes placed into plasmids do not need to have promoters for expression.

    Answer: _____

5.) In which order would you most likely encounter these structures (from first to last) as you approach and enter a gram positive bacterium? (2 pts)

    1. Cell Wall
    2. Plasma Membrane
    3. Capsule

    a.) 1, 2, 3
    b.) 2, 3, 1
    c.) 3, 1, 2
    d.) 1, 3, 2
    e.) 3, 2, 1

    Answer: _____

2
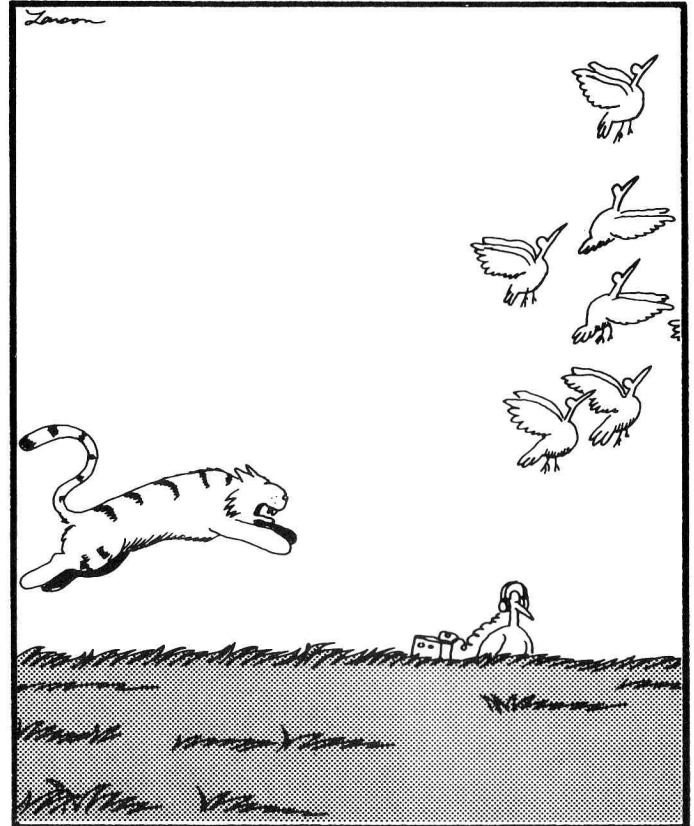
6.) Select two (just 2!) of the following. Describe a major but typical structural difference between the items in each pair you select. (4 pts)

Gram-negative bacteria and gram-positive bacteria.

Bacterial flagella and eukaryotic flagella.

Bacterial chromosomes and eukaryotic chromosomes.

7.) You are trying to insert the gene that codes for a restriction enzyme into a bacterium that normally does not have this gene. If you succeed, and if the gene functions fully in this species, you would expect which of the following will likely result? (2 pts)

    a.) This bacterium will be able to survive and resist bacteriophages better.
    b.) This enzyme will help splice out this gene and move it to other places in the bacterium's genome.
    c.) The bacterium will survive, but the restriction enzyme will cut up any viral DNA sequences already found in the organism's genome.
    d.) The bacterium will die due to its genome being cut by this enzyme.
    e.) The genome of this bacterium will be protected by the methylation of some of the bases at the restriction sites of this enzyme.

        Answer: _____

8.) A linear DNA molecule from a bacteriophage is cloned. The resulting molecules are then successfully cut with combinations of two restriction endonucleases (A and B). The restriction fragments that are produced from each treatment are then separated via gel electrophoresis (at right). Lane 1 has DNA that was treated with enzyme A, lane 2 has DNA that was treated with enzyme B, and lane 3 has DNA treated with both enzymes A and B. Based on these data, what is a likely restriction map for this linear viral DNA molecule? Indicate the relative locations of all essential restriction sites and which enzyme cuts at each site. (4 pts)

Electrophoretic Gel With Viral DNA Fragments
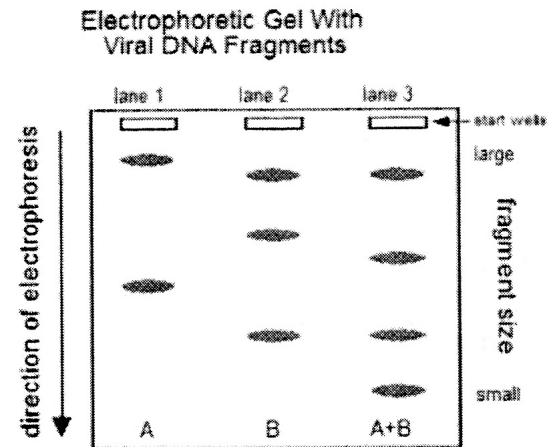
9.) Match the letter of ALL of the items with each technique that uses it. (Some items may be used more than once, others may not be used at all.) (9 pts)

a.) DNA polymerase.   b.) Nucleic acid probes.
c.) deoxyribonucleotides   d.) Gel electrophoresis apparatus.
e.) Nitrocellulose sheets.   f.) dideoxyribonucleotides.

_____ The Sanger method of DNA sequencing.

_____ Southern Blotting.

_____ Polymerase Chain Reaction.
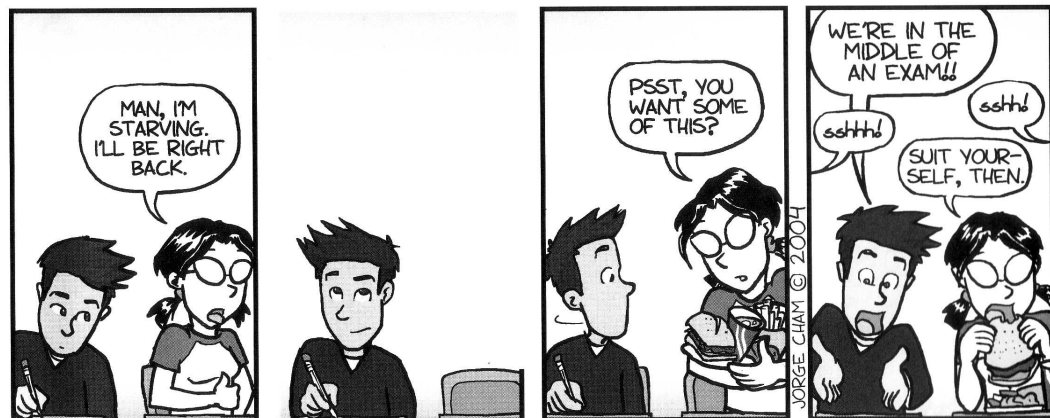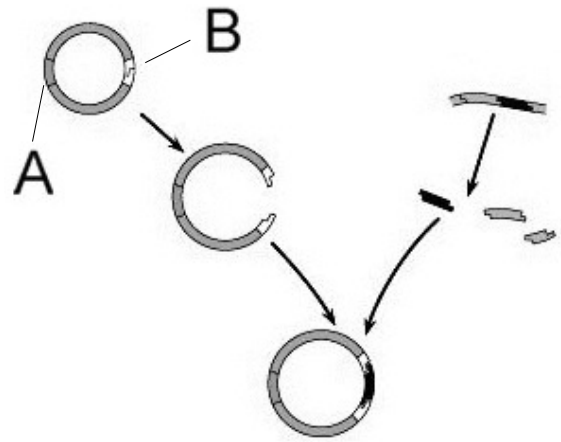
10.) The plasmid in the figure at right has two genes in it (labeled A and B). Describe a distinct and major role of each of these genes in the process of producing a genetically modified organism. (4 pts)

Gene A:
Gene B:

11.) When producing a genomic "library", a eukaryotic organism's DNA is cut up with restriction enzymes and each fragment is inserted into a different plasmid for introduction into a separate bacterium. In contrast the production of a cDNA "library" uses isolated mRNA from an organism. This mRNA is used as a template for reverse transcriptase to guide the creation of complementary DNA (cDNA). Each separate type of cDNA from this mixture of cDNAs is then inserted into a plasmid for introduction into a separate bacterium. Which choice **INCORRECTLY** describes a difference in the information that can be obtained from these two types of "libraries" ? (2 pts)

    a.) Only the genomic "library" will contain the organism's telomeric sequences.
    b.) The contents of a cDNA "library" will be fairly consistent no matter what type of eukaryotic cell supplies the material used to generate it, while the contents of a genomic "library" will vary greatly depending on the type of cell used to produce it.
    c.) Most of the genomic "library" will represent non-protein coding DNA, while most of the cDNA "library" will represent DNA that codes for protein production.
    d.) Information about specific introns is likely to only be obtained from the genomic "library" and not from the cDNA "library".
    e.) The cDNA "library" will have information about just the expressed genes, while the genomic "library" will have information about all the genes in the genome.

    Answer: _____

5

12.) Even if we could somehow identify them all, why would it not be helpful to remove all the genes from our genome that may act as oncogenes later in life? (2 pts)

13.) Multicellular eukaryotes, whose somatic cells have damaged DNA, often use the p53 system to halt those cells in G1 of the cell cycle. This halting of the cell cycle is mainly done so that: (1 pt)

    a.) the damaged section(s) of DNA can be properly transcribed.
    b.) the repair systems have a chance to correct the damaged DNA before entering S phase.
    c.) crossover events with the other chromosomes can replace the damaged DNA.
    d.) this cell's DNA is not used to pass on genetic material to the next generation.
    e.) the cell stays in M phase and good copies of the DNA are segregated into one daughter cell.

    Answer: _____

14.) Which of the following is least likely to be involved in the production of a family of related genes? (2 pts)

    a.) A frame-shift mutation.
    b.) Non-disjunction during mitosis.
    c.) The movement of composite transposons.
    d.) Uneven crossing over during meiosis.
    e.) The movement of a plasmid from a chloroplast into the nucleus.

    Answer: _____
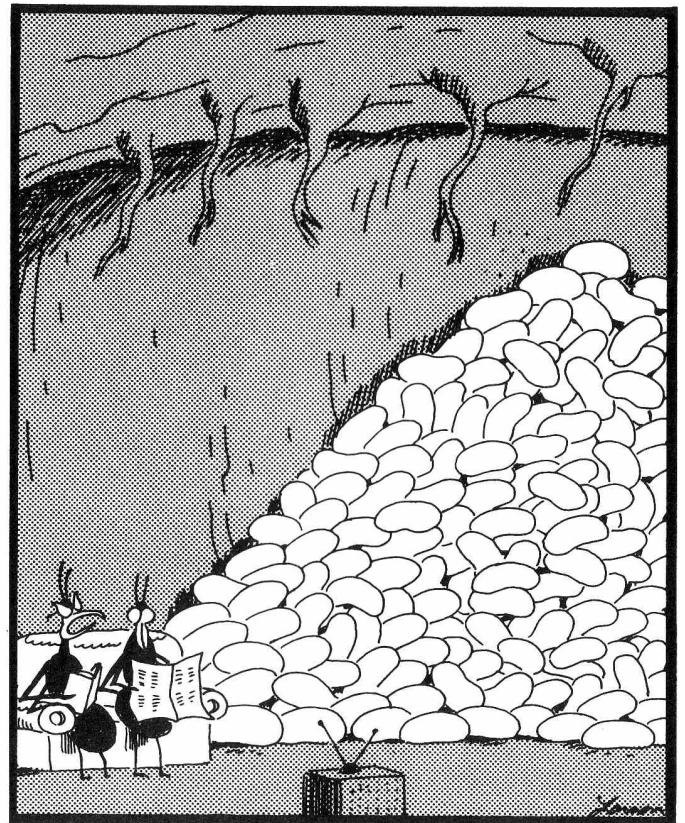
"You know, Vern…the thought of what this place is gonna look like in about a week just gives me the creeps."

15.) Which correctly states a similarity between fungal fertilization and animal fertilization? (1 pt)

     a.) Both involve cells of similar size that must fuse first their cytoplasm and then their nuclei.
     b.) In each case surface ligands and receptors are used to indicate that the two gametes are of the same species.
     c.) Like the animals, fungi have to complete an arrested form of meiosis during fertilization.
     d.) Gamete formation involves the formation of polar bodies in both groups.
     e.) Plasmogamy is typically followed by mitotic divisions before karyogamy is completed.

     Answer: _____

16.) If you arrange the following events of sperm-secondary oocyte interactions of the sea urchin in chronological order, which of these events would be **third** in sequence? (2 pts)

     a.) Acrosomal region undergoes exocytosis.
     b.) Acrosomal process forms.
     c.) Sperm and secondary oocyte membranes merge.
     d.) Enzymes digest jelly coat.
     e.) Bindin and bindin receptors interact.

     Answer: _____

17.) In creating an animal egg and helping to prepare it for fertilization the surrounding maternal nurse cells will send many items to it. Which item is **LEAST** likely to be sent by these maternal cells to the cell that will form the egg? (1 pt)

How the human egg is often deceived.

     a.) Lipids to serve as a food source.
     b.) Proteins that will act as cytoplasmic determinants in the embryo.
     c.) Carbohydrates to serve as a food source.
     d.) Messenger RNAs.
     e.) DNA to help the egg control its gene expression.

     Answer: _____

18.) Select three (just 3!) of the following items. For each item you select, indicate a distinctive role it plays in the human immune system. (3 pts)

Natural Killer Cells:

CD Proteins:

Cytokines:

T cell Receptors:

Thymus:

19.) Describe two things that antibody binding to its antigen can cause to happen, and in what way each event is often a benefit to the organism. (4 pts)

20.) Which is the best description of a difference between helper T cells and cytotoxic T cells? (1 pt)

    a.) Helper T cells confer active immunity; cytotoxic T cells confer passive immunity.
    b.) Helper T cells secrete pore-forming proteins; cytotoxic T cells secrete cytokines.
    c.) Helper T cells have T cell receptors with CD4; cytotoxic T cells have T cell receptors with CD8.
    d.) Helper T cells bind to body cells to determine if they are infected; cytotoxic T cells bind to infected body cells in order to rupture them.
    e.) Helper T cells are each specific for a different antigen; each cytotoxic T cells will attack any infected body cell.

        Answer: _____

21.) During gastrulation in frogs: (1 pt)

    a.) a young embryo creates three distinct tissue layers.
    b.) infolding of one part of a blastula creates the blastocoel.
    c.) the neural crest is created.
    d.) the gray crescent is formed in a frog zygote.
    e.) extra-embryonic membranes are formed.

        Answer: _____

22.) Indicate, with the correct letter, the animal group that each statement best matches. (5 pts)

    F Frog   C Chickens   H Humans

_____ The yolk it has need not be absorbed by the embryo, as it is already in it.
_____ Gastrulation involves a flat disc-shaped structure that sits on top of a large mass of yolk.
_____ An amniote in which several extra-embryonic membranes modify to exchange items with the mother.
_____ This species forms a large and highly vascularized yolk sac.
_____ The grey crescent of the zygote plays a major role in determining a major body axis.

23.) In animals what are two examples of organizing regions, and the development of what animal structure(s) do they each organize? (4 pts)

24.) Which does **NOT** account, at least in part, for the inability of a endothelial cell taken from a mature mammal to display totipotency when grown in cell culture? (2 pts)

     a.) Many of its genes have been covalently modified during maturation.
     b.) A different set of genes are expressed by embryonic cells than are expressed in a mature cell.
     c.) The cell culture medium may not contain compounds that are needed to induce dedifferentiation.
     d.) The cell does not contain the correct set of transcription factors to act as an embryonic cell.
     e.) Cells in the endothelium do not contain the same genes as an embryonic cell.

     Answer: _____
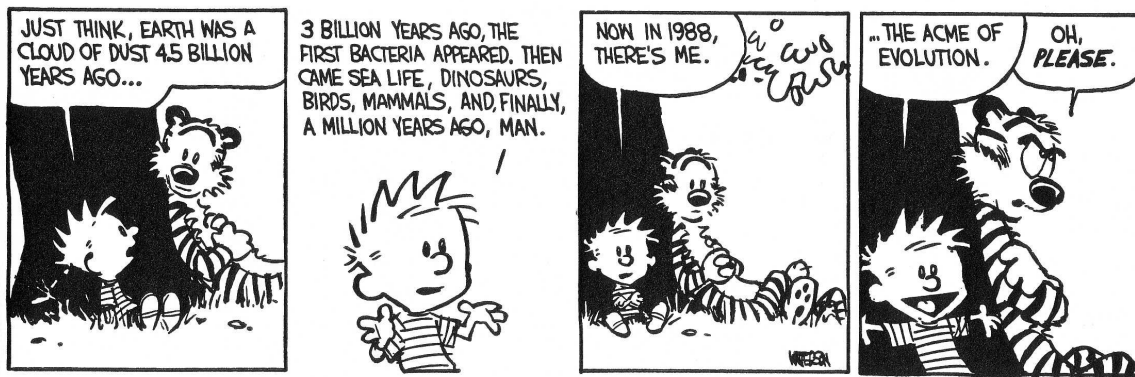
25.) The protein MyoD is a transcription factor and is known to be involved in determination of muscle cells. When this protein was injected into a mature nerve cell it was found to be unable to change this cell into a muscle cell. Which is the most likely reason for this result? (2 pts)

a.) To have an effect MyoD must bind to a receptor on the surface of the cell; not to something on the inside of the cell.
b.) Cytoplasmic injections are known to disrupt cellular determination. Some other means of introducing this protein should be used.
c.) Other essential transcription factors were not present, and in their absence MyoD alone will not have any effect.
d.) The genes needed to become a muscle cell were lost when this nerve cell differentiated.
e.) The protein was likely injected into the cytoplasm, where there are no genes. It had to be injected into the nucleus to have an effect.

Answer: _____

26.) Which one of the following is a CORRECT statement concerning a difference between determination of the anterior in fruit flies and determination of the vulva in nematodes? (2 pts)

a.) Only in the fruit fly example must items pass from one cell to another.
b.) The fruit fly example depends on maternal factors while the nematode example does not.
c.) The arrival of a death signal to the fruit fly zygote results in cell death, while signals arriving to the nematode do not kill it.
d.) The nematode example is entirely intracellular, while the fruit fly example involves items moving through extracellular spaces.
e.) In the case of the nematode vulva there will be differentiation without determination, while a fruit fly's cells will undergo both differentiation and determination.

Answer: _____

27.) In a cladogram, the shared derived characteristics are: (1 pt)

a.) those found in the common ancestor of the specific taxa being compared in the cladogram.
b.) the character states present in the out group for the cladogram.
c.) products of convergent evolution by different evolutionary lineages.
d.) those that appear within one or several of the ingroup taxa of the cladogram.
e.) based solely on DNA sequence analyses.

Answer: _____

28.) In considering vertebrates, the amnionic membrane seen in the amniotes (birds, reptiles, and mammals) is: (2 pts)

    a.) a character useful for distinguishing birds from all other vertebrates.
    b.) a shared ancestral characteristic of vertebrates.
    c.) an example of an analogous structure.
    d.) a shared derived characteristic of the amniotes relative to other vertebrates.
    e.) a characteristic that helps define an outgroup relative to amniotes.

    Answer: _____

29.) Which is **NOT** a condition that should be met if differences in the base pair sequence of a gene found in two species living today are going to be used as a "molecular clock" to estimate when in the past these two species diverged? (2 pts)

    a.) That no versions of this gene were exchanged between the two species over the period of time in question.
    b.) That any selective pressures relating to this gene were similar for each of the two species.
    c.) That enough base pair differences exist so that problems with analogous sequence production can be avoided.
    d.) The mutation rate of one species should not be significantly different from the mutation rate of the other species.
    e.) There must be DNA available from a fossil that is thought to be the common ancestor of these two living species.

    Answer: _____

30.) The sporangia of angiosperms are just the: (3 pts)

    a.) carpels, and sori.   b.) pollen, and archegonia.
    c.) capsules, and megasporophylls.   d.) anther sacs, and nucellus of the ovules.
    e.) flowers, and fruits.   f.) pollen grain, and embryo sac
    g.) filament, and style   h.) stamen, and ovaries.

    Answer: _____

What sexual process happens in all sporangia? _____

31.) Select two (just 2!) of the following sets of items found in flowering plants, and for each that you select describe the major difference between the two items in that set. (4 pts)

Pollen and Sperm	
Embryo and Megagametophyte	
Sepals and Stigma	
Egg and Central Cell	

32.) Based on the following figure, showing the phylogeny of the plant kingdom and its immediate outgroup, indicate at each branching point **an** appropriate shared derived characteristic for that branch (just one for each branch). (5 pts)
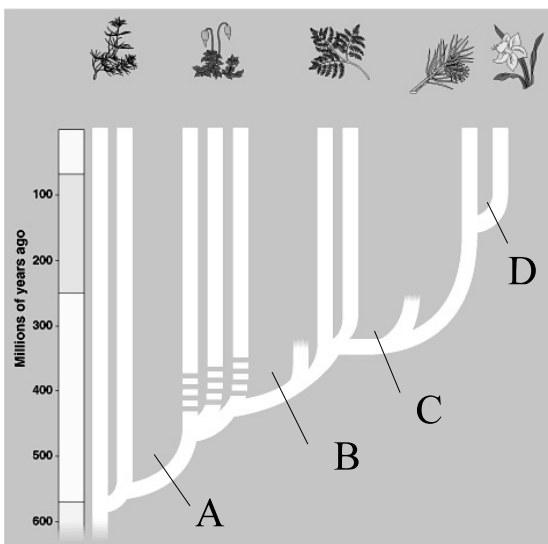
a.)
b.)
c.)
d.)

What is a shared ancestral characteristic that would be expected to be present in the common ancestor of all the lineages shown in this figure?

Optional Bonus Question. Any points earned in an answer to this question will be substituted for up to five missed points out of the seventy that this exam is worth. (Please limit your answer to this side of this page.)

Define transposition as done by transposons. Describe two specific types of transposons.   To carry out transposition what type(s) of enzyme activity is needed? Consider transposons in terms of following situations, and describe the reasoning behind your answers: If a composite transposon moved a homeotic gene, without altering the coding regions of this gene, does this have to alter the development of a multicellular organism? What aspects of bacterial conjugation and of lysogenic viruses are similar to transposons? Overall, is transposition a good or a bad thing to have happen, and why so?

BIO 108   2010
Exam #2   Proposed Answers:

Below are proposed answers for the questions in this exam.

Be aware that for many of these questions other answers exist that may also be acceptable either for full or partial credit.

___

1.) C.

2.) (Only two answers were needed here.)
        RNA viruses:
RNA replicase. This enzyme uses RNA as a template to guide the formation of new RNAs, and this process leads to new viral RNAs being created.
        Retroviruses:
Reverse transcriptase. This uses RNA as a template to guide the formation of viral DNA for insertion into the host genome.
        Temperate viruses:
These viruses use a restriction endonuclease to cut the host DNA so that the viral DNA can be inserted into it.

3.) E.
4.) E.
5.) C.

6.) (Only two answers were needed here.)
        Gram-negative and gram-positive bacteria:
Gram negative bacteria have an extra outer membrane, that is outward of the cell wall. The gram positive bacteria do not have this outer membrane.
        Bacterial flagella and eukaryotic flagella:
The bacterial flagella are extruded extracellular secretions that anchors to the cell at membrane spanning complexes. The eukaryotic flagella are cellular structures that include the cell membrane and some of the cell's cytosol.
        Bacterial chromosomes and eukaryotic chromosomes.
Bacterial chromosomes tend to be circular, while eukaryotic chromosomes are typically linear.

7.) D.
8.)

A   B   B

9.)

A, B, C, D, F	The Sanger method of DNA sequencing.
B, D, E	Southern Blotting.
A, B, C	Polymerase Chain Reaction.

10.)

Gene A:

When the phenotype coded for by this gene shows up in a cell that previously did not have it, then we know that the cell took up this plasmid. So this tells us if the plasmid was taken up by the cell.

Gene B:

When the phenotype coded for by this gene is NOT displayed, then something has altered this gene. This indicates that it is likely that some new recombination of genetic material occurred and that a recombinant plasmid was taken up by the cell.

11.) B.

12.) *Since many oncogenes code for items we need to have during early development, if we removed all the oncogenes from our genome then our offspring would likely die, or have unusual development, early in life.*

13.) B.
14.) A.
15.) B.
16.) D.
17.) E.

18.) (Only three answers were needed in this case.)

Natural Killer Cells:

These are cells that induce apoptosis in body cells that fail to display the proper surface items, such as MHCI.

CD proteins:

These are proteins (CD4 and CD8) associated with the T cell receptors which assist in their interacting with the appropriate major histocompatibility complexes (MHCII and MHCI respectively) on other cells.

Cytokines:

These are small organic molecules, typically peptides, that are secreted by a variety of immune system cells. The act as intercellular signals between cells of the immune system, enhancing their activities upon detecting these signals.

T cell Receptors:

These are membrane spanning proteins on T cells that detect items displayed on the surface of other cells that are from inside of those cells. These receptors vary from cell to cell, giving each T cell a specific antigen target that it can detect.

Thymus:

An organ in the body where many cells of the immune system, mainly T cells, mature and are checked to be sure that they do not react to self-antigens.

19.)
        The antibody could bind to and so cover the surface of a virus. This covering of bound antibodies could inhibit the virus' ability to enter and infect its target cell.
        Antibodies in our body fluids that have bound their antigen can be detected by various phagocytotic cells (i.e. macrophages). This removes the antibody and its bound antigen from the body fluid, and makes it possible to display the antigen to cells of the T cell system.

20.) C.
21.) A.

22.) The letters down the statements should be: F, C, H, C, F.

23.)
        The dorsal lip of the blastula of frog embryos helps to organize the point of invagination during gastrulation. This helps to form the anus end of the digestive tract.
        The zone of polarizing activity (ZPA) in the development of chicken wing limbs helps to organize the anterior/posterior axis of these limbs.

24.) E.
25.) C.
26.) B.
27.) D.
28.) D.
29.) E.

30.) D.   The sexual process that is done is: meiosis.

31.) (Only two answers were needed in this case.)
        Pollen and sperm.
Pollen is a multicellular item, while sperm are unicellular.
        Embryo and megagametophyte.
The embryo is a young, diploid, organism. While the megagametophyte is a haploid organism.
        Sepals and stigma.
Stigma are the area at which pollen must arrive if proper pollination is to be achieved. Sepals play no direct role in pollination.
        Egg and Central Cell.
The egg cell has one haploid nucleus. While the central cell typically has two nuclei, each of which is haploid.

32.)   a.) Alternation of generations.
        b.) Vascular tissue.
        c.) Heterospory.
        d.) Flowers.

        They all have chloroplasts that have chlorophylls A and B.

Monday, August 9   Name: _____
        Points earned: _____ = _____ for course.
                                        145
Please keep your answers within the spaces provided. Good luck!

1.) Which of the following is an INCORRECT statement? (2 pts)

    a.) In the life cycle of the green alga *Chlamydomonas* sp. the products of meiosis could
        either be spores and go on to do mitosis, or be gametes and go on to do
        fertilization.
    b.) In the life cycle of mushrooms (Basidiomycetes) the events of plasmogamy produce a
        dikaryotic cell that can do many mitotic divisions before fertilization is finally
        completed.
    c.) The typical plant life cycle includes the production of spores, that grow into
        gametophytes, as part of their sexual reproduction.
    d.) Some eukaryotic species lack any multicellular generation(s) in their life cycles.
    e.) In some animal species, such as the hydra, the products of meiosis can undergo
        "budding" and produce additional multicellular offspring without immediately
        forming a zygote.

    Answer: _____

2.) Check each and every one of the following statements that is INCORRECT with regards to the
principle of segregation. (7 pts)

    _____ It always involves the separation of alleles from two different parents.
    _____ It occurs just during gamete formation.
    _____ Since a haploid cell lacks different alleles, it does no segregation when it divides.
    _____ One type of error in the process of segregation is called non-disjunction.
    _____ Segregation typically occurs during anaphase I of meiosis, and only sometimes
          occurs during anaphase II of meiosis.
    _____ Each of the chromosomes that are separated as sets during segregation must have
          sister chromatids in it.
    _____ The principle of segregation accounts for all the features attributed to the principle
          of independent assortment.

3.) Which is an aspect of modern genetics that Gregor Mendel did NOT describe as a result of his studies using crosses of various peas? (2 pts)

   a.) One of the two versions of each gene is passed down to each gamete from an ancestor who was a heterozygous individual.
   b.) The version of one gene that ends up in a gamete is not influenced by what versions of other genes are given.
   c.) When two different versions of a gene are present in an individual one version may be dominant in effect to the other.
   d.) Genetic factors exist as distinct particles, and need not blend in effect with each other.
   e.) The influence that some genes have on the phenotypic expression of other genes.

   Answer: _____

4.) Phil and Karen are going to be married and wish to have children. Both of them has a brother who has phenylketonuria (PKU) which is an inherited disease seen when an individual has the homozygous recessive genotypic state for a gene. Neither Phil nor Karen have PKU, nor do their parents. Given this: (5 pts)

   a.) What is the probability that Phil is a carrier for PKU? _____

   b.) What is the probability that a child of Phil and Karen will have PKU? _____

5.) A trihybrid pea is heterozygous for pod color (Gg), height (Tt), and for seed shape (Rr). In the space below draw the chromosomes that would hold these genes as they might appear during Metaphase I of meiosis if these genes are physically linked or physically unlinked, assuming no crossing over. In each drawing indicate the location of all the alleles for each of these genes. (6 pts)

Unlinked condition:	Linked condition:
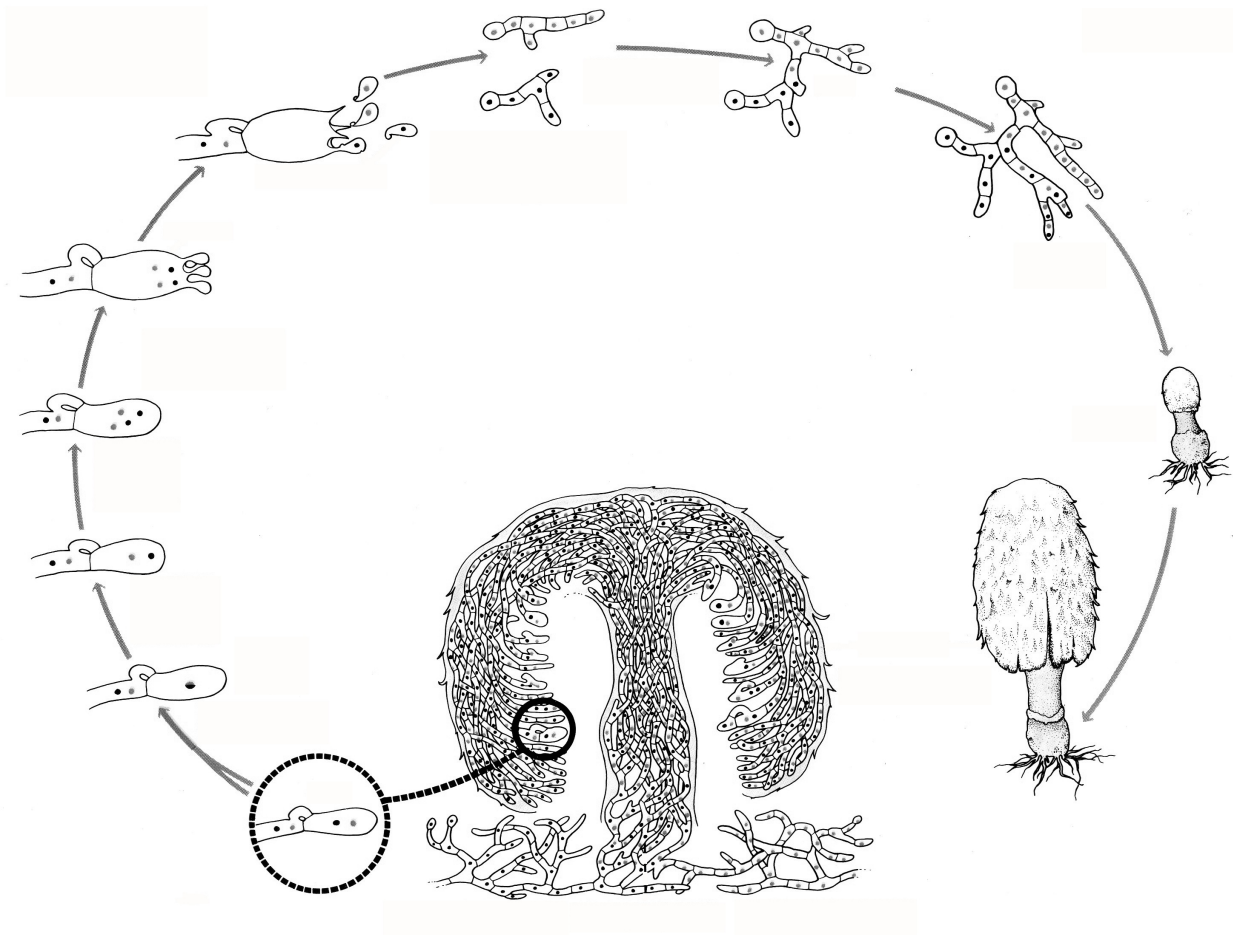
6.) Refer to the above figure in answering the following questions. (5 pts)

a.) This figure shows aspects of the life cycle of which group of fungi? (circle your choice)

    Basidiomycota   Ascomycota   Zygomycota

b.) Label, with a number and a line drawn to it, an item in the figure that best illustrates each of the following structures or processes.

    1.) An item that this fungus disperses into the air.
    2.) A cell that has just completed karyogamy.
    3.) A hyphal strand that will be in direct contact with the soil.
    4.) A dikaryotic cell.

3

7.) One thing Griffith showed was that exposing non-pathogenic bacteria to items from pathogenic bacteria can change the phenotype of the bacteria. (5 pts)
a.) What are three items that the non-pathogenic bacteria could take up that would likely alter its phenotype (at least for a while)?

b.) Other than influencing the phenotype of the cell what else does Griffith's experiment say this transforming agent must be able to do once it is in these new cells?

8.) *E. coli* grown for many generations in a complete growth medium containing ^{14}N were transferred to a complete growth medium containing ^{15}N long enough for two complete rounds of DNA replication to occur. The DNA from these cells was then extracted and separated by ultracentrifugation. Which best describes the expected results? (2 pts)

    a.) One high-density and one low-density band.
    b.) One intermediate-density band.
    c.) One high-density and one intermediate-density band.
    d.) One low-density and one intermediate-density band.
    e.) One high-density band.

    Answer: _____

9.) In producing their arginine synthesis mutants, Beadle and Tatum used a mutation inducing treatment that most likely resulted in a change in which part of the fungus they studied? (2 pts)

    a.) The promoter region of a gene.
    b.) The mRNA.
    c.) The introns of a gene.
    d.) The ribosomes in the cytosol.
    e.) Several of the amino acids in the fungus.
    f.) The spliceosomes in the nucleus of the fungus.

    Answer: _____

4

Oh my goodness!.. Vinnie's mutating!

Embarrassing moments at gene parties

10.) A feature unique to ribosomal RNA compared to other RNAs is that it: (1 pt)

    a.) has many folds giving it a characteristic three-dimensional shape.
    b.) catalyzes the formation of peptide bonds.
    c.) lacks any distinct 3' end.
    d.) holds in itself the anticodon sequences.
    e.) is able to splice out introns from mRNA.

    Answer: _____

11.) A feature that is unique to transfer RNA compared to other RNAs is that it: (1 pt)

    a.) has a specific amino acid bound to its 3' end.
    b.) has the codon sequence exposed at one of its folds.
    c.) is exported from the nucleus of eukaryotes.
    d.) must be transcribed from a gene.
    e.) does not itself code for the sequence of amino acids in a polypeptide.

    Answer: _____

12.) In *Serratia* sp. the production of prodigiosin pigment protects the bacteria from ultraviolet light. There is a long biochemical pathway involved in the production of prodigiosin. Given this, which of the following is LEAST LIKELY to be the case in this bacterium? (2 pts)

    a.) The genes coding for the enzymes in this pathway are all together in one operon.
    b.) Prodigiosin acts as an inducer.
    c.) A repressor protein is involved in control of expression of the genes in this pathway.
    d.) When present above a certain concentration, prodigiosin acts as a corepressor.
    e.) When not enough prodigiosin is present, genes for its production will be used in transcription.

    Answer: _____

CHARLES DARWIN SLIDING INTO THIRD WITH HIS REALIZATION THAT THE FITTEST SURVIVE

5

13.) Eukaryotic gene expression is said to be dependent on the formation of complexes of transcription factors. Which one of the following items is found in bacteria and is most similar to the eukaryotic transcription factors in function? (1 pt)

      a.) supercoiling of the DNA
      b.) sigma factor of the RNA polymerase
      c.) distal control elements of gene expression
      d.) translational control of gene expression
      e.) enhancers of gene expression

      Answer: _____

14.) Does viral assembly illustrate a feature of the principle of segregation, yes or no? Explain the reasoning behind your answer. (3 pts)

15.) Which of the following is typically an asexual process? (1 pt)

      a.) Transduction of a bacteria gene into a new species of bacteria.
      b.) The formation of spores by a fern.
      c.) The movement of an F^+ plasmid through a conjugation tube.
      d.) Division of *E. coli* cells.
      e.) The transformation of a bacillus cell.

      Answer: _____

16.) Which is a CORRECT statement about prokaryotes? (1 pt)

      a.) Like other life forms, many prokaryotes display behaviors such as taxis.
      b.) Prokaryotes do not have any type of sexual recombining of their genetic material.
      c.) There are no examples of cellular specialization found amongst the prokaryotes.
      d.) All prokaryotes lack any form of internalized membranes with specialized functions.
      e.) Prokaryotes are heterotrophic, none of them are autotrophic.

      Answer: _____

17.) To operate well it would be best if a probe that is intended to hybridize with a specific protein-coding gene in a species' genome had which of the following features? (Mark all that apply.) (4 pts)

    _____ It should be double stranded.
    _____ It has a chemical group that fluoresces when exposed to a certain type of light.
    _____ It is the same nucleotide sequence as the region to be found.
    _____ It is DNA.
    _____ It is a circular strand of RNA.
    _____ It is complementary to a section in an intron-coding section of the gene.
    _____ It is complementary to a conserved part of the promoter of the gene.
    _____ It is complementary to a part of an exon-coding section of the gene.

18.) When trying to insert a new gene into a eukaryotic organism it is typical to include a well known gene (sometimes called a "reporter gene") in the DNA that will hold the new gene. Which choice best describes a valid reason for using reporter genes? (1 pt)

a.) Larger pieces of DNA are more likely to be taken up by the organism than are smaller pieces.
b.) The reporter gene may code for a phenotype which is easier to detect than the gene you wish to introduce.
c.) The gene you introduce will not be replicated if it lacks a reporter gene associated with it.
d.) The reporter gene can act as a promoter for the expression of the gene you wish to have expressed.
e.) The use of reporter genes introduced along with your new gene allows you to determine whether these two genes are linked or not.

Answer: _____

19.) In order for retrotransposition to occur, what are two enzyme activities that are needed? State the function of each enzyme you name. (4 pts)

7

20.) Fill in the following blanks with the most appropriate words or phrases. (4 pts)
To ensure species specificity animals that use external fertilization typically have a receptor

located in the plasma membrane of their _____ that detects a ligand

located in the _____ of their secondary oocytes. Another specific receptor is

located in the plasma membrane of the _____ that detects a ligand

that is displayed on the surface of the _____.

21.) Answer the following questions concerning the display of items on a human MHCII protein.
(3 pts)
       Name a type of cell likely to display items on MHCIIs: _____
       What other specific type of cell would be activated if it binds well to the above cell's MHC?

               _____
       What is the name of the protein that specifically binds an item displayed on an MHCII?

               _____

22.) If you put the following terms in order, as they first appear in development of a normal frog
zygote into an embryo, which would occur THIRD in order? (2 pts)

       a.) Invagination.   b.) Blastula.
       c.) Cleavage.   d.) Gray Crescent.
       e.) Archenteron.

          Answer: _____

23.) The process of differentiation seen in most animal cells typically involves which one of the
following? (1 pt)

       a.) The excision and loss of sections of DNA that will not be needed for the cell's
           functions.
       b.) Apoptosis by most of the cells created in the early embryo.
       c.) Only signals that arrive early during development.
       d.) The presence of cytoplasmic or extracellular inducers that ultimately alter gene
           expression.
       e.) The irreversible methylation of sections of RNA.

          Answer: _____

The figure represents the relatedness of species A-E, and indicates times 1-5 in the past.   Refer to it for the next two questions.

24.) A point in the past when species D and B had their most recent common ancestor would be at time number: (1 pt)

a.) 1. b.) 2.
c.) 3. d.) 4.
e.) 5.

Answer: _____

25.) According to the information in this figure, which of the following pairs of species seem most closely related to each other? (1 pt)

a.) A and C.   b.) E and D.
c.) C and B.   d.) D and C.
e.) B and D.

Answer: _____

26.) Which trait is LEAST likely to be helpful in adapting pines for life in dry habitats? (2 pts)

a.) Retention of the megaspore in the
      sporangium.
b.) A thick waxy cuticle covers its leaves.
c.) The possession of vascular tissue in the
      sporophyte generation.
d.) Dispersion of seeds rather than spores.
e.) Production and accumulation of toxins to
      repel herbivores.

Answer: _____
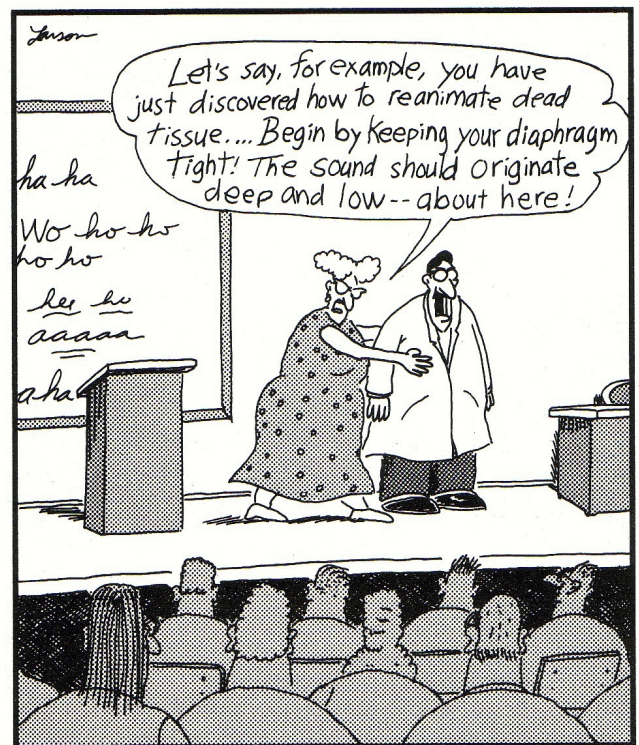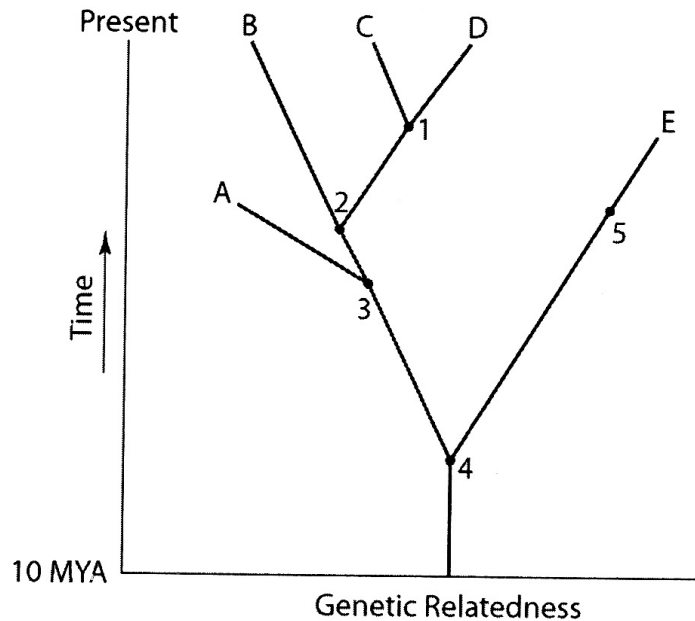
In their final year, all research science students are required to take one semester of Maniacal Laughter.

9

27.) Refer to the images at the right in answering the following questions. (7 pts)

a.) The male organism shown in these images is called the:

_____

b.) Label one example of each of the following items with a line and the appropriate number.

      1.) The micropyle.
      2.) Integuments.
      3.) The ovarian wall.
      4.) An egg.
      5.) A triploid nucleus

c.) Given just what is shown in these images is this flower:

    Perfect or   Imperfect ? (circle your choice)

28.) The geological ideas of gradualism and uniformitarianism contributed what to biologists' thinking of speciation? (1 pt)

      a.) The fossils discovered in geological strata represent ancestors to many of today's living species.
      b.) The earth is so ancient that even very slow processes can lead to very large changes over the time available.
      c.) Variation in the local soil contributes to the variation found in species living in that area.
      d.) Any speciation that occurs must be a slow and gradual process.
      e.) That there is a uniform rate of formation of new species, and a uniform rate of extinction of existing species.

      Answer: _____
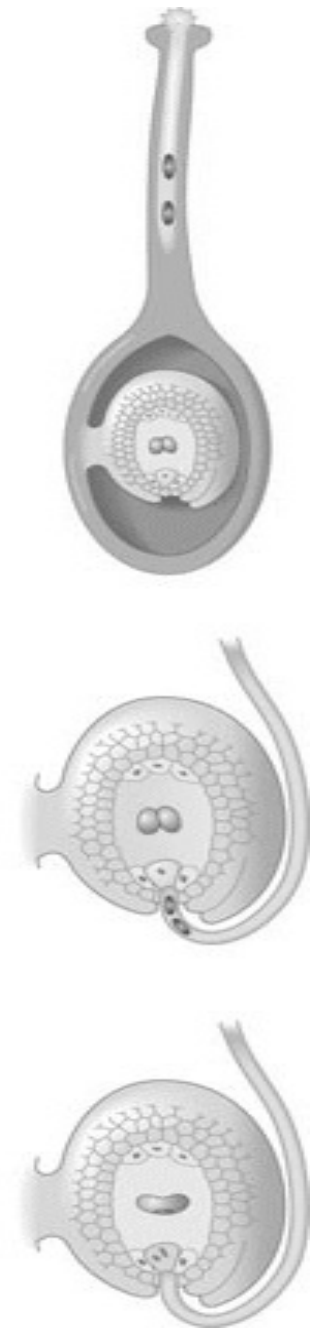
29.) Identify three items that are necessary for Darwin's model of speciation, as driven by the mechanism of natural selection, to operate. (3 pts)

| |
| |
| |

30.) Previous to Charles Darwin's model of biological evolution biologists had considered the idea that speciation could occur, but most of this thinking was rejected for a variety of reasons. Which is NOT an argument likely to have been given by biologists before the 1850s for rejecting models for the formation of new species? (2 pts)

a.) No new species had been observed to have formed by the proposed models.
b.) New varieties within a species might form, but no new species were expected.
c.) The Earth was thought to have existed for only tens of thousands of years; too short a time to allow for the formation of all the known species of life.
d.) Previous analyses, such as Malthus' essay on population, showed that resources would seldom be limiting resulting in little or no pressure for changes in the characteristics of a species.
e.) Each species was thought to reflect a fixed "type" with variations amongst individuals being due mainly to the local conditions of growth.

Answer: _____

31.) Which is NOT a structure found in typical snails (Phylum Mollusca)? (1 pts)

a.) A radula.   b.) A muscular foot.
c.) A calcareous exoskeleton.   d.) A gastrovascular cavity.
e.) A mantle.

Answer: _____

32.) The "rule-of-thumb" is that an animal with an open circulatory system will tend to have a relatively low metabolic rate and be sessile. (6 pts)

a.) Identify one invertebrate animal that is consistent with this stereotype, and describe how it manages to carry out gas exchange.

b.) Identify one invertebrate animal that is an exception to this stereotype, and describe how it manages to carry out gas exchange.

33.) Which statement BEST contrasts a gastrovascular cavity and a complete digestive tract?
(1 pt)

a.) The gastrovascular cavity occurs in diploblastic organisms, while complete digestive tracts are found in triploblastic organisms.

b.) The gastrovascular cavity develops from the blastocoel, while the complete digestive tract develops from the archenteron.

c.) The complete digestive tract is a coelom, while the gastrovascular cavity is not a coelom.

d.) The gastrovascular cavity can only carry out partial digestion, while a digestive tract can carry out complete digestion.

e.) A complete digestive tract has a distinct mouth and anus, while a gastrovascular cavity does not.

Answer: _____

Well, Randy, you've gone and spoiled your dinner, haven't you?

34.) Identify the following features of the Ames test. (3 pts)
    a.) What is the observable response that this test shows to a researcher?

    _____

    b.) In order for the above response to be observed, what must happen in the bacterial cells that are used in this test?

35.) In order for mosquitoes to display attraction to malaria-infected humans the mosquitoes must: (1 pt)

    a.) be infected with the malarial parasite.
    b.) be unable to find any uninfected humans for a blood meal.
    c.) have a specific receptor, or set of receptors, activated in their olfactory organs.
    d.) be able to taste the presence of compounds from the malarial parasite in the blood of
        the infected humans.
    e.) need to use the compound from the malarial parasite as an essential nutrient.

        Answer: _____

36.) You carry out a bioassay and find that treating with the sample you have results in a response that is below threshold for the assay. What should you do with your sample before doing this bioassay again? (2 pts)

13

37.) The following species concepts have been used to various degrees with regards to eukaryotes, but which would you argue would be most useful in trying to designate species of prokaryotes? Circle the concept you choose, and describe the reasoning behind your answer. (3 pts)

      Biological   Morphological   Phylogenetic   Genetic

Reasoning:

38.) Which of the following is most likely to ensure that a new eukaryotic species will be formed in the least amount of time? (2 pts)

    a.) Members of a species are separated into distinct populations.
    b.) Viable offspring are made through mating with another species.
    c.) The rate of mutation of the DNA in the cells of an animal increases greatly.
    d.) Some members of the species have significantly different morphologies from other members of the species.
    e.) The extent of natural selection applied to members of a species is consistent over time.

       Answer: _____

39.) To account for the rarity of transitional forms in the fossil record biologists who favor punctuated equilibrium would argue that: (1 pt)

    a.) the time it takes for speciation to occur is relative short, making fossils of transitional forms rare.
    b.) speciation tends to occur in areas where the formation of fossils is very unlikely.
    c.) fossils of transitional forms should be common, they just have not been found yet because no one is looking for them.
    d.) most speciation is due to allopolyploidy events and so no intermediate forms should be found in the fossil record.
    e.) during speciation events in the past there were more opportunities for allopatric speciation than are present today.

       Answer: _____

14

40.) If a population is in Hardy-Weinberg equilibrium we would expect all of the following statements to be true except for one. Which one statement is the exception? (2 pts)

      a.) The allele frequencies in the population will change from one generation to the next.
      b.) The net rate of mutation in this population is so low as to be negligible.
      c.) Whatever variation exists amongst individuals in the population, it will not alter
          their chances to successfully reproduce.
      d.) No phenotypic trait will be found that increases the chance of being chosen as a
          mate.
      e.) Individuals of the population never move out of the population.

        Answer: _____

41.) Coloration in the peppered moth is determined by a single gene with two alleles, with one showing complete dominance to the other. Dark moths are homozygous dominant or heterozygous, light moths are homozygous recessive. In a population of 100 moths, you determine that 64 of the moths are dark. Then, according to the Hardy-Weinberg rule, the expected frequency of the dominant allele is ____. (2 pts)

      a.) 0.8   b.) 0.64
      c.) 0.6   d.) 0.4
      e.) 0.36

        Answer: _____

42.) In which of the following ways is natural selection most likely to alter the allele frequencies in a population? (2 pts)

      a.) During meiosis only certain versions of each chromosome that carry the most fit allele
          are passed to the cells that will be formed.
      b.) There can be an expansion of the gene pool as new types of alleles are added to the
          population.
      c.) The location of the gene in the species' genome can be altered, moving it to a place
          that is better able to induce that gene's expression.
      d.) New copies of the allele are made in the genome, giving it a higher allele frequency in
          the species' genome.
      e.) Only the surviving and reproducing individuals are able to pass their alleles to the next
          generation.

        Answer: _____

43.) Which is an INCORRECT ecological interaction for the example given? (2 pts)

a.) Mutualism - An impatiens flower gives nectar to a bee and the bee acts as a pollinator for the flower.
b.) Commensalism - By mimicing a corpse, a flower attracts flies that end up being covered with the flower's pollen before moving to another flower.
c.) Parasitism - An orchid mimics a female wasp and so tricks a male wasp into acting as a pollinator for it and costing the wasp time it could have used seeking a real mate.
d.) Mutualism - To gain their services as pollinators a fig provides a chamber with flowers in which a wasp can lay its eggs, and later the larvae feed on some of the flowers' ovules.
e.) Predation - While acting to pollinate a flower species, a beetle eats some of its pollen grains.

Answer: _____

44.) Which statement is INCORRECT? (1 pt)

a.) Dispersion requires establishment of a reproducing line in a new location.
b.) Fertilization involves the union of two haploid cells to produce a diploid cell.
c.) Most plants make their gametes by mitosis, not by meiosis.
d.) Flowering plants typically use a structure containing a young sporophyte for dispersal.
e.) Pollination is the way that flowering plants engage in sexual reproduction.

Answer: _____

45.) Which was LEAST likely to have been present during the running of, or made by, the Miller/Urey apparatus as it was originally used? (1 pt)

a.) Methane (CH_4).
b.) Simple amino acids (i.e. glycine, alanine).
c.) Small sugars and formaldehyde.
d.) Polypeptides.
e.) Short hydrocarbons.

Answer: _____
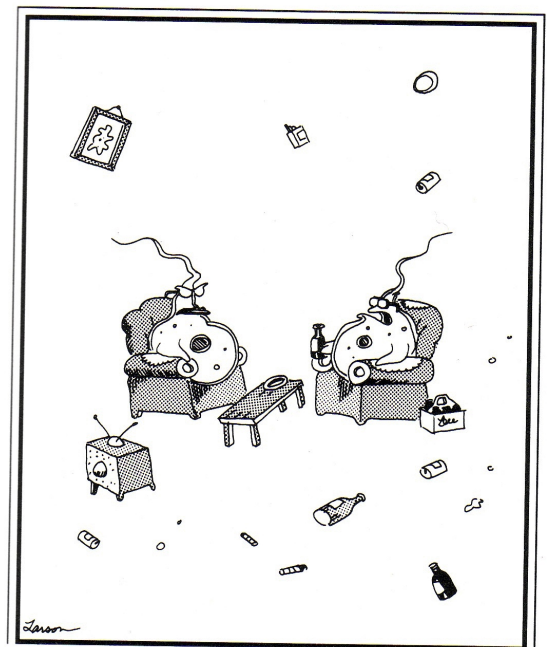
"Hey! I got news for you, sweetheart! …
I *am* the lowest form of life on earth!"

16

46.) Describe and distinguish the processes of replication and reproduction. In your answer note for each process an example of a non-living item that illustrates that process. (6 pts)

47.) Which one of the following is NOT a feature that would have to be accounted for by a model of the abiotic origin of life in order for that model to be credible?   (1 pt)

a.) A means to compartmentalize an early cell so that it has a distinct interior separate from the external environment.
b.) A demonstration that each of the four classes of organic matter found in life can be formed in the absence of life.
c.) A means to have a separate origin for each of the three major domains of life.
d.) The ability of simple polymers formed abiotically to act as catalysts.
e.) The ability of simple polymers to act as a template for their own replication.

Answer: _____

48.) While climbing up mountains, one observes transitions in biological communities. Which factor is most likely to account for this observation? (1 pt)

a.) A rise in the availability of nutrients at higher elevations.
b.) A drop in average temperatures with a rise in elevation.
c.) A rise in the amount of solar radiation experienced due to a thinning of the atmosphere.
d.) A drop in precipitation with elevation.
e.) Regions at the top of mountains are geologically young and many species have not had sufficient time to disperse to these areas.

Answer: _____

49.) The Sahara desert, the Gobi desert, and the southwestern desert of the United States are all close to the Tropic of Cancer in the northern latitude. The common occurrence of deserts in these areas is best accounted for by: (1 pt)

       a.) the curvature of the Earth.
       b.) the greenhouse effect.
       c.) a rise in human population leading to an increased rate of desertification.
       d.) the rain shadow effect.
       e.) poor soil nutrient conditions in these areas limit the growth of vegetation there.

          Answer: _____

50.) One proposal to try to deal with global warming involves positioning a set of large flat satellites between the Earth and the Sun, so that the intensity of light that reaches the Earth is lowered. What is this likely to do to the trade winds? (2 pts)

       a.) Those trade winds that go towards the equator would tend to be stronger, while those that go towards the poles would become weaker.
       b.) This might weaken the trade winds.
       c.) This would have no affect on the trade winds.
       d.) The trade winds would stay the same strength, but shift differently with the seasons.
       e.) The trade winds near to the poles would become stronger, but those near the equator would tend to weaken.

          Answer: _____

51.) According to the logistic growth equation: (2 pts)

       a.) when the population size is close to zero the population will add the highest number of people per time.
       b.) as the population size becomes close to the carrying capacity the per capita birth rate will rise.
       c.) the death rate will be the highest when the population size is much lower than the carrying capacity.
       d.) the population growth rate will approach zero as the population size approaches the carrying capacity.
       e.) a population can never undergo anything close to exponential growth.

          Answer: _____

52.) What are two assumptions that are made when using the mark-and-release technique to try to estimate the number of individuals of an animal species in an area? (2 pts)

| |
| |

53.) What two features of the age structure of a population might help you decide whether the species is using an R-selected strategy or a K-selected strategy? Describe the reasoning behind your answer. (6 pts)

| |

54.) The competitive exclusion principle states that: (1 pt)

    a.) two species in a community do not exist together in exactly the same niche for long.
    b.) two species cannot exist together in the same area.
    c.) competition between two species always causes extinction or emigration of one of the species.
    d.) competition in a population promotes survival of the best-adapted individuals.
    e.) species that compete usually coevolve.

       Answer: _____

55.) The concept of an ecological "niche" of a species includes which of the following? (Check all that apply.) (2 pts)

_____ The size and age structure of the population of the species.
_____ The interactions, both positive and negative, the species has with other species.
_____ The maximal intrinsic rate of growth of the species.
_____ The amount of available water that the species needs each day to live on land.

56.) A beaver builds a dam and creates a small pond. The beaver then eats much of the trees around the pond it made. From the point of view of a fish that lives in the pond, what ecological relationship does the fish species have with the beaver? Explain the reasoning behind your answer. (4 pts)

57.) Unlike gross productivity, net productivity takes into account: (1 pt)

    a.) only the energy contained in items actually eaten by that trophic level.
    b.) the efficiency with which the energy will be used by the organism.
    c.) the total surface area over which the production is occurring.
    d.) the energetic costs of growth and maintenance.
    e.) the influence of limiting nutrients on the rate of production.

    Answer: _____

Now put the wallet on the counter!
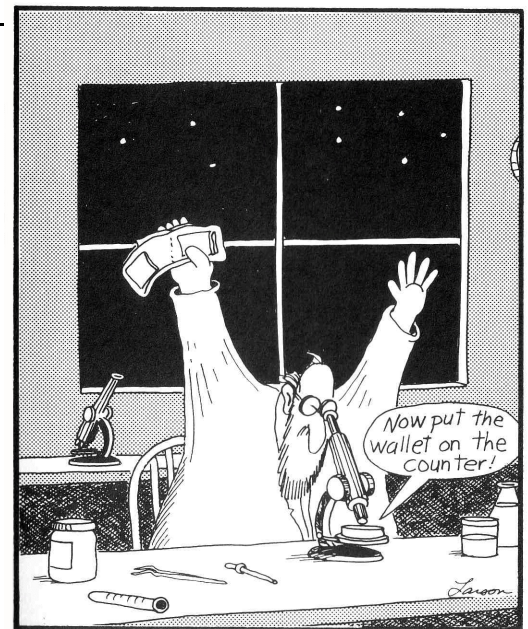
Working alone, Professor Dawson stumbles into a bad section of the petri dish.

58.) Tropical forest soils contain comparatively low concentrations of mineral nutrients because: (1 pt)

    a.) the existing standing biomass of tropical forests is very small.
    b.) tropical soil microorganisms do not break down organic matter very rapidly.
    c.) nutrients in the soil, or released from the break down of organic matter, are taken up rapidly by existing plants.
    d.) nutrient cycling is very slowly in the tropics since the soil holds minerals tightly.
    e.) the high tropical temperatures destroy many of the nutrients.

        Answer: _____

59.) In the peat bogs found in the arctic tundra there is currently a high amount of accumulated carbon in organic form. This accumulation is mainly due to: (1 pt)

    a.) a low rate of energy incorporation by the primary consumers in these bogs.
    b.) the lack of food needed by detritivores to live in this accumulated organic matter.
    c.) a very high rate of carbon fixation by the moss in the bogs.
    d.) the absence of primary consumers in this ecosystem.
    e.) the low activity of detritivores and decomposers due to the low temperatures of these bogs.

        Answer: _____

60.) Fill in the following blanks with the most proper word or phrase. (2 pts)

Primary producers use energy in the form of _____ to drive the conversion of inorganic compounds into organic compounds. Primary consumers and detritivores then typically power their conversions of forms of matter by obtaining energy via the process of

_____ done on items they obtain from the primary producers.

61.) Which of the following is LEAST likely to occur when a population drops below its minimum viable population (MVP) size? (1 pt.)

    a.) The population will undergo genetic drift.
    b.) Due to inbreeding the fertility of members of the population will drop.
    c.) The carrying capacity of the area for that species will rise.
    d.) The effectively reproducing members of the population will tend to produce most of the offspring.
    e.) The population size will likely continue to decline.

        Answer: _____

62.) Which of the following would most rapidly increase the genetic diversity of a threatened local population? (1 pt)

a.) Capture all remaining individuals in the population for selected captive breeding, followed by reintroduction of their offspring to the wild.
b.) Establish a reserve that protects this population's habitat.
c.) Introduce new individuals of this species from other populations into this threatened population.
d.) Sterilize the least fit individuals in the population.
e.) Control the population sizes of predators of this threatened population.

Answer: _____

63.) Which is LEAST likely to be a problem with attempts to protect a migratory species from extinction? (1 pt)

a.) Areas must be preserved that are critical to their migration even if the species is only in those areas for a few days out of each year.
b.) Often the migratory path of a species is not well known.
c.) Identifying critical areas in their migratory path, versus those areas seldom used, is difficult.
d.) Often it is not enough to reserve areas in their migratory path, their food supplies in those areas must also be identified and protected.
e.) Even with enough food and range space, many species lack the ability to have their population size grow after declining below the carrying capacity.

Answer: _____

22

Optional Bonus Question. Any points earned in an answer to this question will be substituted for up to five missed points out of the one hundred and forty five that this exam is worth. (Please limit your answer to this side of this page.)

Trade winds typically bring clouds from the west across the north/south aligned mountain ranges of the western United States. Describe how the eastern and western faces of these mountains would likely compare in terms of: The type of ecosystem on each face. The relative amount of energy that will likely flow through these two ecosystems. Which area is more likely to have more species-species interactions? Relate your description to what you would expect to happen if a population of a species was divided between these two mountain faces in terms of what selective pressures each subpopulation would likely encounter over time. Would this more likely lead to allopatric or sympatric speciation, and how long might such speciation take to occur?

BIO 108   2010
Final Exam   Proposed Answers:

Below are proposed answers for the questions in this exam.

Be aware that for many of these questions other answers exist that may also be acceptable either for full or partial credit.

_____

1.) E.

2.)
_X_	It always involves the separation of alleles from two different parents.
_X_	It occurs just during gamete formation.
_X_	Since a haploid cell lacks different alleles, it does no segregation when it divides.
_____	One type of error in the process of segregation is called non-disjunction.
_X_	Segregation typically occurs during anaphase I of meiosis, and only sometimes occurs during anaphase II of meiosis.
_X_	Each of the chromosomes that are separated as sets during segregation must have sister chromatids in it.
_X_	The principle of segregation accounts for all the features attributed to the principle of independent assortment.

3.) E.
4.)   a.) 2/3   b.) 1/9
5.)

Unlinked condition: Linked condition:

1

6.) Basidiomycota

7.)
a.)
    DNA,
    RNA,
    Proteins.
b.) It must be passed on
to daughter cells.

8.) C.
9.) A.
10.) B.
11.) A.
12.) B.
13.) B.

14.)
Yes. Each new viron
that is made must have a
full set of the viral genome in it. Those virons that don't achieve this suffer the consequence of
violating the principle of segregation and will either lack certain genes or have too many copies
of other genes.

15.) D.
16.) A.
17.)
        _____ It should be double stranded.
        _X_   It has a chemical group that fluoresces when exposed to a certain type of light.
        _____ It is the same nucleotide sequence as the region to be found.
        _X_   It is DNA.
        _____ It is a circular strand of RNA.
        _____ It is complementary to a section in an intron-coding section of the gene.
        _____ It is complementary to a conserved part of the promoter of the gene.
        _X_   It is complementary to a part of an exon-coding section of the gene.

18.) B.

19.)
        A DNA endonuclease is needed to cut the ends of the transposons and the location
where it will be inserted.
        A DNA ligase will be needed to make new phosphoester bonds at the location where the
transposon is inserted.

20.) ..... sperm..... .....extracellular matrix....   ....secondary oocyte.... .....sperm....
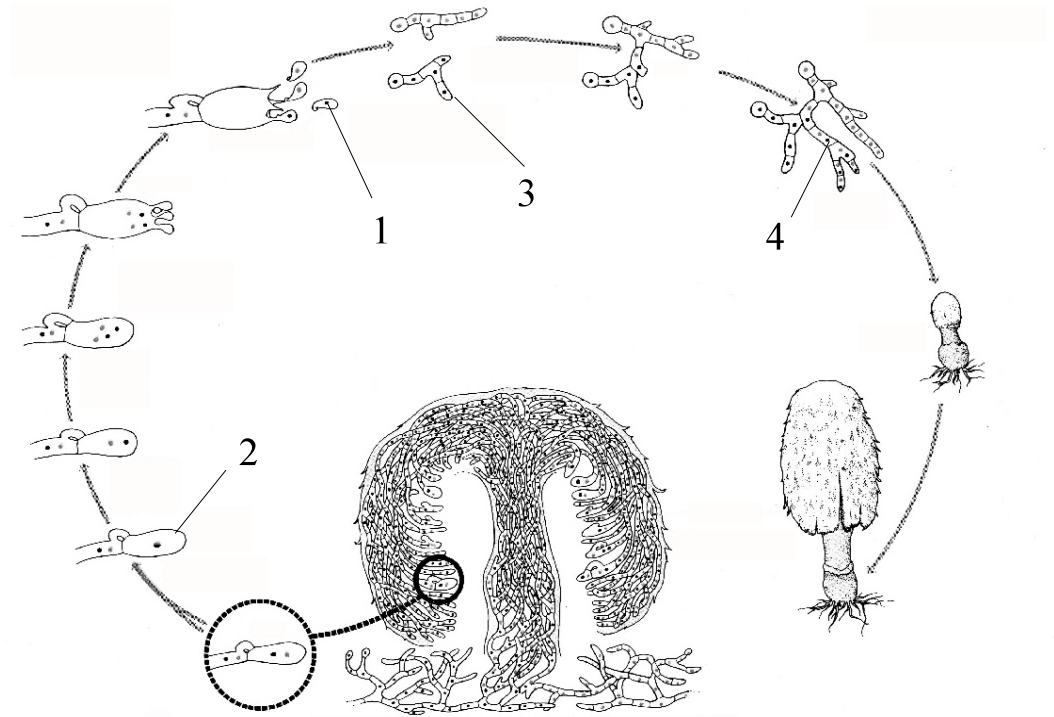
21.)   B cells
         Helper T cells
         T cell receptor

22.) B.
23.) D.
24.) B.
25.) D.
26.) E.

27.)   a.) pollen grain
         b.) (see figures at right)
         c.) imperfect

28.) B.

29.)
         There must be variation between individuals in the population.
         The variation must be heritable.
         The variation must lead to differences in the ability of individuals to
               survive to reproduce.

30.) D.
31.) D

32.)
         a.) Clams. They pull water over their vascularized gills and move the
               gases through their open circulatory system through their body
               tissues.
         b.) Grasshoppers. They have air filled trachea through which gas can
               move and diffuse up close to nearly all the cells of their body.
               Thus the gases do not need to move through the circulatory
               system at all.

33.) E.

34.)   a.) The bacteria survive and form colonies on the minimal media plates.
         b.) These bacteria had a mutated form of a gene whose wildtype product was needed to
               make an essential amino acid.   To live, these bacteria had to have a back-
               mutation to revert to the wildtype version of this gene.

35.) C.

36.) The sample should be concentrated so that the items in it are at a higher concentration for
         the next attempt.
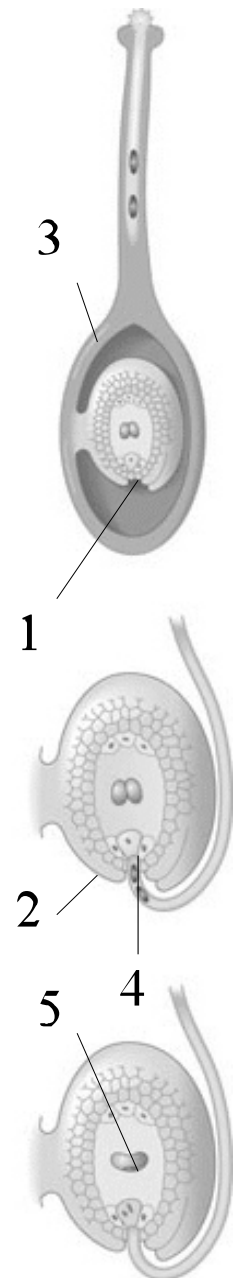
37.) (This question is mostly graded on the basis of the reason given.)
 Genetic.
 We can isolate the genomes of various prokaryotes, sequence them, and compare them
  to determine relatedness.

38.) B.
39.) A.
40.) A.
41.) D.
42.) E.
43.) E.
44.) E.
45.) D.

46.)
 Replication involves the formation of new copies of molecular structures. One example
of this is seen in the growth of crystals. Each new section of the crystal forms a structure that is
informed by the previous sections.
 Reproduction involves the formation of new individual structures rather than the growth
of one structure. Thus the shattering of the above crystal would make many new crystals from
the one original one and might be argued to be like reproduction.

47.) C.
48.) B.
49.) A.
50.) B.
51.) D.

52.)
 That there was enough time between when the marked individuals were released before
they were recaptured for them to mix well with the population in the area.
 That capturing of the individuals to mark them did not alter the chances of capturing
them again.

53.)
 An R-selected species would have a high rate of population growth. This would be seen
in its population age structure as very few older individuals and a great many young individuals.
 In contrast a K-selected species would tend to have a slower rate of population growth,
and so its population age structure would have a higher proportion of older individuals and a
lower proportion of younger individuals in it.

54.) A.

55.)

         _____ The size and age structure of the population of the species.
         X   The interactions, both positive and negative, the species has with other species.
         _____ The maximal intrinsic rate of growth of the species.
         X   The amount of available water that the species needs each day to live on land.

56.)

        This relationship could be called a commensalistic one. The beaver neither gains benefits or suffers costs from the fish that live in the pond it built. But the fish benefit greatly from the work of the beaver to build and maintain the pond.

57.) D.
58.) C.
59.) E.

60.) ....light... .... respiration....

61.) C.
62.) C.
63.) E.

CHAPTER 1 – CELL DIVISION—GENETIC CONSEQUENCES

Jon C. Glase and Kuei-Chiu Chen

Revised by Mark A. Sarvary, June 2010

LABORATORY SYNOPSIS

In this series of activities, you will study the behavior of chromosomes in the two processes that organisms use to make new cells: mitosis and meiosis. Mitosis is the generalized cell division procedure used by unicellular eukaryotes during asexual reproduction and by multicellular organisms needing to produce additional cells for growth and maintenance. Meiosis is a special form of cell division used to produce cells that will serve in a sexual role as gametes or nonsexually as spores in the life cycle of an organism. Since the genes, which determine the hereditary characteristics of organisms, are located on the chromosomes in the nucleus, the activities of chromosomes during mitosis and meiosis have important consequences for the study of genetics.

After studying prepared onion root tip slides and projected slides of mitosis in plant and animal cells, you will use poppet bead models of chromosomes to simulate mitosis and meiosis and consider their genetic implications. In particular, you will explore the two major principles of genetics: the principle of segregation and the principle of independent assortment, and consider their application in studies of the genetics of haploid and diploid organisms. After this laboratory, you should be able to accomplish the following conceptual and procedural objectives.

LABORATORY OBJECTIVES

Conceptual

1. Name and describe the important events of mitosis.

2. Name and describe the important events of meiosis.

3. Describe important similarities and differences between mitosis and meiosis.

4. Understand the timing of mitosis, meiosis, and fertilization in the general life cycle of sexually reproducing organisms.

5. Differentiate between the following pairs of terms: chromatid and chromosome; single- and double-chromatid chromosomes; homologous and nonhomologous chromosomes; haploid and diploid.

6. State Mendel's principle of segregation and explain its physical basis in meiosis and mitosis.

7. State Mendel's principle of independent assortment and explain its physical basis in meiosis.

8. Apply the principle of independent assortment to determine the number of genetically different classes of cells that an individual of known genotype can produce through meiosis.

9. Explain how construction and use of the Punnet square have their physical basis in meiosis and the random combination of gametes occurring in fertilization.

Procedural

1. Simulate mitosis and meiosis with poppet bead chromosome models.

2. Demonstrate Mendel's two principles of inheritance by using poppet bead models to simulate meiosis, gamete production, and genetic crosses.

READING ASSIGNMENT

Read this entire chapter before coming to the laboratory.

For additional information on chromosomes, cell division, and life cycles see Chapter 14 in *Biology* (Campbell et al., 2008).

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

1. How many DNA molecules are present in a pair of homologous, double-chromatid chromosomes?

2. For a single gene, how many alleles are present in a pair of homologous, double-chromatid chromosomes?

3. Why are there two divisions in meiosis but only one in mitosis?

4. A haploid cell can undergo mitosis, but not meiosis. Why?

5. What is meant by the statement, "crossing over is equal and reciprocal"?

6. During which meiotic stage does segregation of the genetic material occur?

7. During which meiotic stage does independent assortment of unlinked genes occur?

TODAY SET-UP FOR THURSDAY'S (JULY 15TH) LAB ON BIOCHEMICAL PATHWAYS

Read p. 53-55 in Chapter 3 of the lab text to familiarize yourself with the procedure.

IMPORTANT GENETIC CONCEPTS

The genetic information that is passed from one generation to the next resides in the DNA molecules within cells. DNA is a polymer of nucleotides and the sequence of nucleotides within DNA determines the sequence of amino acids in the polypeptides and proteins that are coded for by the DNA. The proteins, both enzymes and structural proteins, determine the appearance and functional characteristics of the cell — the genetic traits that are passed from one generation to the next. **Chromosomes** consist of DNA molecules located within the nucleus of the cell. Chromosomes can be a single DNA molecule (a **single-chromatid chromosome**) or, after DNA replication, two DNA molecules (a **double-chromatid chromosome**). A chromatid is a single molecule of DNA, but also includes various proteins that are attached to the DNA molecule and help to regulate the processes of DNA replication and transcription. In preparation for cell division, all single-chromatid chromosomes become double-chromatid chromosomes as their DNA is replicated. During DNA replication, each DNA molecule is copied, so immediately after replication, the chromatids of a double-chromatid chromosome are genetically identical. The chromatids of a double-chromatid chromosome, called **sister chromatids**, are attached to each other at a special region of the DNA molecule called the **centromere**.

A **gene** is a portion of a chromosome that determines the inheritance of a specific genetic trait. The gene's location is called its **locus**. The nucleotide sequence present at the gene locus is called an **allele**. The different alleles of a gene code for different variations of a polypeptide. Each polypeptide is expressed at the cell and organism level as a particular form of the trait. The **genotype** of a cell or organism is a listing of symbols representing alleles of the genes being considered. The **phenotype** of a cell or organism is a description of the outward expression of alleles in terms of structural and functional characteristics.

In species with more than one chromosome, each chromosome type differs in size, shape, centromere position, and the genes included on the chromosome. For example, the fungus *Sordaria fimicola* has seven different chromosome types; humans have 23 different chromosome types. Cells can be described by their **ploidy** or **n number**. Ploidy is the number of sets of chromosomes that are present in the cell, where each set includes one of each of the different types of chromosomes. A **haploid** cell has one set of chromosomes (1n) and, therefore, has one allele for each gene. A **diploid** cell has two sets of chromosomes (2n) and, therefore, has two alleles for each gene. Diploid cells are usually formed by the process of **fertilization**, which combines two haploid cells so that both sets of chromosomes come to reside within the same nucleus.

In a diploid cell, the chromosomes of the same type from the two chromosome sets are called **homologous chromosomes**. Homologous chromosomes look alike and have the same genes, but, depending on the two parental haploid cells, can have different alleles for the genes present. Genes that are on the same chromosome are called **physically linked genes**. Genes on chromosomes that are not homologous (**nonhomologous chromosomes**) are **physically unlinked genes**.

Life forms with a sexual mode of reproduction show an alternation between haploid and diploid stages called a **life cycle** (Figure 1.1). In some species the haploid stage is multicellular and the diploid stage is unicellular (most fungi); in some the haploid stage is unicellular and the diploid stage is multicellular (most animals); and in some both stages are multicellular (most plants) or unicellular (some algae). Two types of cell division are intimately connected with life cycles: **mitosis** and **meiosis**. Either haploid or diploid cells can undergo mitosis because mitosis maintains the ploidy condition of the parent cell in the daughter cells. Mitotic cell division occurs to produce the multicellular stages of the life cycle, if present, or to produce additional unicellular individuals. Meiosis reduces the ploidy of the daughter cells to one-half that of the parent cell, so only diploid cells can undergo meiosis. The two events that divide life cycles into their haploid/diploid phases are fertilization, which combines two haploid cells to produce a diploid cell, and meiosis, which produces haploid cells from a diploid cell.

Today you will compare mitosis and meiosis and consider the implications of meiosis and fertilization for the study of genetics. Please note that most of the concepts we discuss in this chapter apply to eukaryotic cells. Prokaryotes have different mechanisms in dividing up the genetic content in the cell. We will not include them in the discussion.
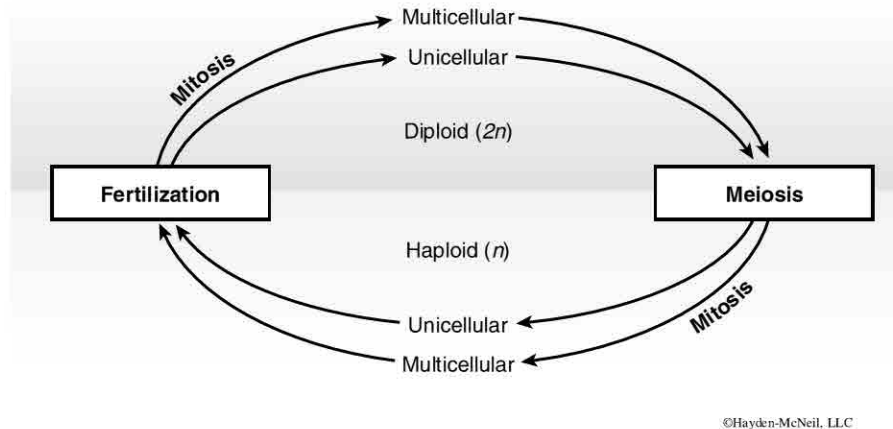
Figure 1.1. Generalized life cycle of organisms with sexual reproduction. Different species are multicellular or unicellular in the haploid or diploid portions of their life cycles.

Many kinds of cells follow a cycle of reproductive and nonreproductive phases called the **cell cycle**. The period of the cycle when the cell is actually dividing and its chromosomes are visible is called **mitosis**. The non–mitotic period of the cell cycle is called **interphase**. Replication of the cell's DNA occurs during interphase.

Interphase

Chromosomes are not apparent in the nuclei of cells in interphase because the DNA molecules are in the form of unlooped, thread-like strands called **chromatin**.

MITOSIS

Mitosis, followed by **cytokinesis** (division of the cytoplasm), is a process that produces two genetically identical cells. The chromosomes of cells beginning mitosis each consist of two genetically identical sister chromatids. Mitosis separates the sister chromatids of the double-chromatid chromosomes and distributes a single-chromatid chromosome of each type to both of the new cells. Thus, the cells formed by mitosis get a complete complement of genes, the alleles are the same for each gene in the two cells, and the ploidy number has not changed (a haploid cell produces two new haploid cells; a diploid cell produces two new diploid cells). Before a cell produced by mitosis can divide again it must replicate its DNA so that its single-chromatid chromosomes again become double-chromatid chromosomes.

For convenience, mitosis is divided into 4 major stages (prophase, metaphase, anaphase, and telophase), but remember that it is a continuous process.

❑*   Each student should obtain a compound microscope and a slide of mitosis in onion (*Allium* sp.) root tip. As you study these slides, search for examples of specific stages using the low power objective. When a cell is found, center it, and switch to the high power objective for detailed study. Identify the structures discussed in the following sections and use the terms mentioned in the text to label the left hand portion of Figure 1.2.

❑   Locate the region of the root tip where cells are dividing. In this slide, interphase nuclei appear reddish, nucleoli are darker red, and cytoplasm is green. Nucleoli are the sites of ribosomal RNA synthesis.

How many nucleoli do you see in each cell? _____

Prophase

The helical DNA molecules of each chromatid of the double-chromatid chromosomes now begin to form supercoils; as a result, the chromosomes shorten and thicken and become visible. Prophase begins when the chromosomes become apparent as discrete bodies. By the end of prophase, the nuclear membrane and nucleoli have disappeared from view. The chromosomes are visible as discrete bodies, and a system of microtubules called a **mitotic spindle** envelops the chromosomes. The spindle converges at the two ends, or poles, of the cell. At this stage, can you see any evidence that chromosomes consist of two sister chromatids joined by a centromere?

❑   Locate cells in this stage of mitosis. The nucleoli have disappeared and chromosomes are visible but still restricted to the spherical nuclear region. The mitotic spindle is beginning to form.

Metaphase

In this stage, chromosomes move to the center of the cell and line up with their centromeres positioned precisely along the equatorial plane of the cell. Some of the mitotic spindle microtubules are attached to the centromeres.

❑   Find cells in which chromosomes are lined up in the center of the cell. You should be able to see the individual chromatids of some chromosomes. The mitotic spindle is fully formed.

Anaphase

This stage begins when the centromeres of sister chromatids uncouple and the individual chromatids (now single-chromatid chromosomes) move toward the poles of the cell. Spindle microtubules may cause this movement.

❑   Find cells in which two separate groups of single-chromatid chromosomes are visible. You can see that the centromeres move first, with the chromosome arms dangling behind.

---

* Text preceded by a ❑ is instructions for a procedure to be done by you.
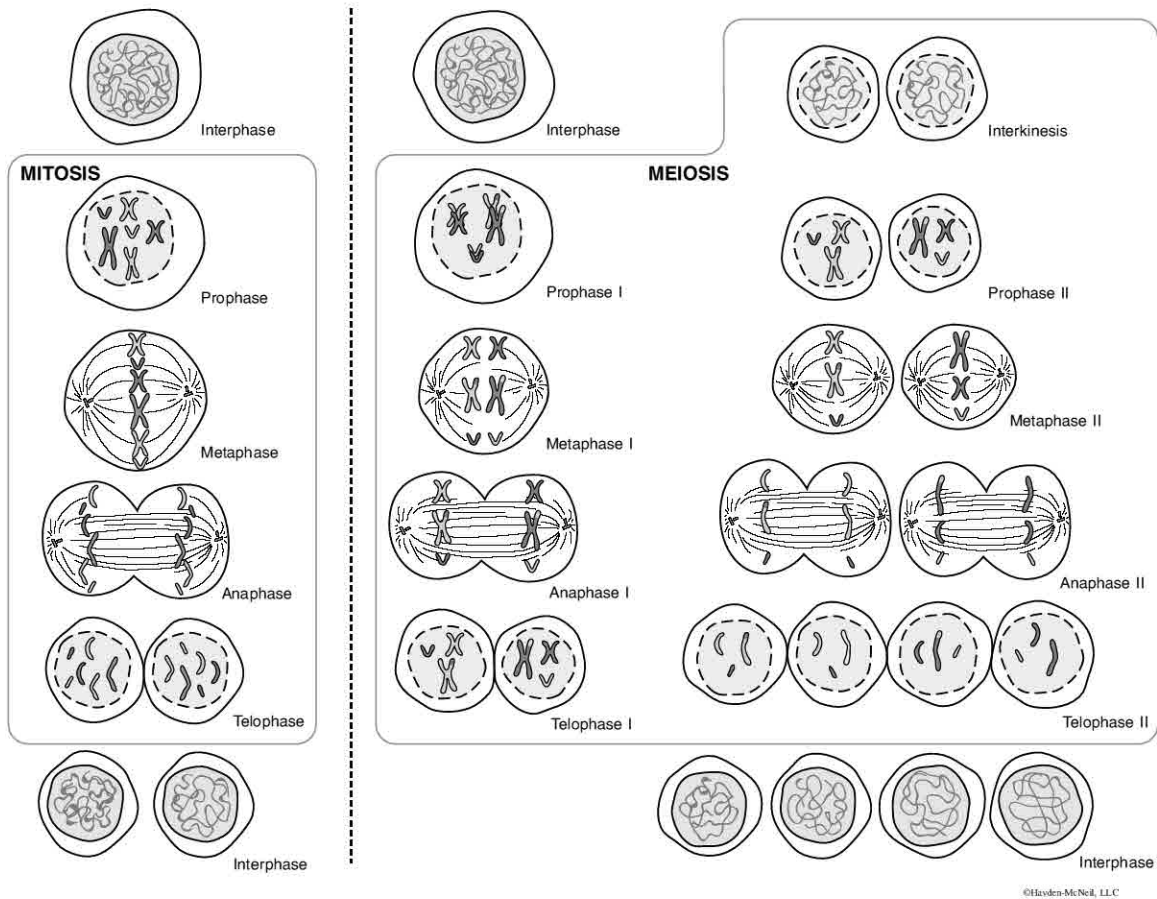
Figure 1.2. Mitosis (left) and meiosis (right) in an animal cell. Note: in meiosis a crossover has occurred between one pair of homologous.

Telophase

This stage is the reverse of prophase. The nuclear membrane reforms around each set of chromosomes, which fade from view as their DNA molecules begin to uncoil and lengthen. In many species, cytokinesis (division of the cytoplasm) occurs during telophase. In animals, cytoplasm is divided by an inward pinching of the cell membrane (called the **cleavage furrow**) in the area of the equatorial plane until two separate cells are formed. In plants, a **cell plate** composed of pectin and calcium pectate forms in the middle of the equatorial plane and spreads outward. The cytoplasm on each side secretes cellulose onto the cell plate, producing the primary cell wall and effecting a separation of the two new cells. When cytokinesis is complete and mitosis has ended, each cell enters interphase of the cell cycle.

❑   Find cells in which two groups of chromosomes are restricted to spherical regions where nuclear membranes are forming. Observe the various stages of cell plate formation, which separates cells in telophase into pairs of daughter cells.

Summary of Mitosis

During the synthesis stage of the cell cycle, each chromosome is exactly copied to form the two identical chromatids of a double-chromatid chromosome. Therefore, when mitosis begins the cell contains double-chromatid chromosomes and each chromosome consists of two identical sister chromatids. During anaphase, the two chromatids of each chromosome separate, and are moved to opposite sides of the cell. The two cells produced at the end of mitosis are genetically identical. Each cell contains an identical chromosome for each of the chromosomal types typical of the species.

❑ To review what you have learned about mitosis, your lab instructor will project slides of plant and animal cells in various stages of mitosis and cytokinesis.

? [*] You have been viewing projected microscope slides of onion root tips and an embryonic stage in a fish called the Whitefish (*Coregonus* sp.). Why would you expect these cells to be undergoing mitosis?

MEIOSIS

Meiosis is a special kind of cell division that reduces the number of chromosomes in the four haploid nuclei that are formed to one half the number found in the diploid parent nucleus. Further, the meiotic process ensures that each new nucleus receives one complete set of chromosomes.

As in mitosis, the DNA replicates during the preceding interphase, so when the diploid nucleus enters meiosis all its chromosomes are double-chromatid chromosomes. Meiosis involves two successive divisions of the nucleus. The first division reduces the number of chromosomes from $2n$ to n by separating the two homologous chromosomes of each chromosome type and distributing them to different nuclei. The second division is very similar to mitosis in that it splits double-chromatid chromosomes to form single-chromatid chromosomes. To differentiate which meiotic division is being discussed, a I or II will follow the name of each stage. In many species, a cytokinesis follows meiosis I and II, resulting in four haploid cells.

❑ Your lab instructor will project slides of meiosis in male and female parts of flowers of the lily. Identify the structures discussed in the following sections and ask any questions you may have about these slides. Using the terms mentioned in the text, label the right hand portion of Figure 1.2.

Note: in the male part of the flower, meiosis of a cell called a microsporocyte produces four cells that develop into four pollen grains. In the female part of the flower, meiosis of a cell called a megasporocyte produces four haploid nuclei, three of which disintegrate. The remaining cell divides mitotically several times to produce a structure called an embryo sac. After fertilization the embryo sac becomes part of the seed. Thus, in the life cycles of higher plants, both haploid and diploid stages are multicellular (see Figure 1.1). You will consider the life cycle of higher plants later in the semester.

---

[*] Text preceded by a **?** is a question to be considered and answered by you.

Prophase I

As the chromosomes shorten and thicken, they stain more readily and become visible. Homologous chromosomes, each composed of two chromatids joined by coupled centromeres, come together in a process called **synapsis**. During synapsis, the homologous chromosomes lie alongside each other so that their centromeres are adjacent and the banding patterns match. The chromatids of each chromosome intertwine with those of its homologue. While intertwined, these non-sister chromatids often fuse at certain points along their length. These fusion points are called **chiasmata** (singular **chiasma**). Chiasmata result when sections of adjacent, non-sister chromatids become detached from their original chromosome and switched to the other homologue. This equal and reciprocal exchange of genetic material between non-sister chromatids is called **crossing over**. Although the exchanged sections contain the same genes, the allelic forms of the genes may be different in the two switched pieces. The genetic implications of crossing over will be considered later in this chapter. As the nuclear membrane disappears and the microtubules become organized into a spindle, homologous chromosome pairs move as units to the equatorial plane.

Metaphase I

During metaphase I, the homologous chromosomes line up on opposite sides of the equatorial plane. Each homologous chromosome pair lines up independently of all other pairs. As we shall discuss shortly, the autonomous behavior of homologous chromosome pairs is the physical basis for Mendel's **principle of independent assortment**.

Anaphase I

The separation of the homologous chromosome pairs occurs during this phase. Homologues of each pair separate and move to opposite ends of the cell. Unlike anaphase in mitosis, centromeres do not divide and the chromosomes remain double-chromatid chromosomes throughout the first division. Anaphase ends with one member of each pair segregated into a group of chromosomes near each pole of the spindle. The separation of homologous chromosomes is the physical basis of Mendel's **principle of segregation** to be considered later. Cytokinesis usually begins during late anaphase. Each of the two new cells receives a haploid set of double-chromatid chromosomes.

Telophase I

This stage is not always present, since in some organisms the two groups of chromosomes immediately undergo the second meiotic division. If telophase is present, chromosomes uncoil and fade from view as the nuclear membrane reforms, and a short interphase called **interkinesis** occurs.

Prophase II

The second division of meiosis is much like a mitotic division. Toward the end of prophase II, the double-chromatid chromosomes move to the equatorial plane.

Metaphase II

Chromosomes are arranged on the spindle's equatorial plane with their centromeres attached to separate microtubules. The spindle that forms during metaphase II is usually at a right angle to the orientation of the spindle during the first division.

Anaphase II

Sister chromatids separate as their centromeres divide, and the newly formed single-chromatid chromosomes move to the two poles of the spindle.

Telophase II

This stage results in the formation of four groups of chromosomes, which, after cytokinesis, are contained in independent cells. The four cells resulting from meiosis are called a **tetrad**, and each cell contains *n* number of chromosomes. (The four chromatids of synapsed homologous chromosomes are also called a tetrad.) Thus, meiosis starts with one cell with 2*n* double-chromatid chromosomes and produces four cells, each with *n* single-chromatid chromosomes.

MODELING MITOSIS AND MEIOSIS

You will now use strands of poppet beads as chromosome models to simulate the events of mitosis and meiosis in a diploid cell with <u>three pairs</u> of homologous chromosomes (*n* = 3). The different homologous chromosomes are represented by varying the size of the chromosome (number of beads) and the placement of the centromere. Keep your chromosomes simple, with 3-6 beads per chromosome. For each chromosome type, the parental origin of the chromosomes is designated by different bead color (red or blue). Therefore, homologous chromosomes will have the same number of beads with the same centromere placement, but one will be red and the other blue. Double-chromatid chromosomes are represented by two strands of the same color connected by special "centromere" beads. Work in groups of 3 to 4 students and complete Table 1.1 as you do this simulation. For the anaphase results in Table 1.1, record the composition of only one of the two groups resulting from chromosome separation.

In order to complete the simulation you also need to know what is meant by a **group** of chromosomes at various stages in cell division. Between metaphase and anaphase of mitosis and meiosis chromosomal arrangement in a cell goes through drastic transition by separating a single cluster of chromosomes into two separate clusters. Each cluster, named a **group**, will show a distinctive number of chromosomes, chromatids, and homologous pair of chromosomes in relation to their number in the original cell.

❑   Obtain a container with the beads you will need to simulate mitosis and meiosis concurrently.

❑   Begin the simulation at interphase **prior** to the DNA replication stage of the cell cycle. Assume that the cell is **diploid** and that there are three different types of chromosomes **(2*n* = 6)**.

Per cell, &

?   How many different chromosome types must be built? _____

?   Are they single chromatid chromosomes or double chromatid chromosomes? _____

?   How many strands of beads will you need? _____

?   How many strands should consist of red beads and how many should be blue? _____

❑   Simulate DNA replication by attaching an identical strand of beads to each single-chromatid chromosome.

❑   Now begin the mitotic and meiotic stages, using Figure 1.2 as a guide. Compare the behavior of
      chromosomes in the two processes and the chromosomal condition of the cells that result.

Table 1.1. Results of a simulation of mitosis and meiosis in a diploid cell with *n* = 3 using poppet bead chromosome models. For anaphase, record the composition of only one group of the two separating groups of chromosomes.

	Number of Chromosomes per group& per cell&		Number of Chromatids per group& per cell&		Number of Homologous Pairs per group& per cell&	
Mitosis metaphase						
Mitosis anaphase						
Meiosis metaphase I						
Meiosis anaphase I						
Meiosis metaphase II[1]						
Meiosis anaphase II[1]						

[1] Assume that a complete cytokinesis follows meiosis I.

?   Does synapsis occur in mitosis? If so, during which stage?

_____

?   Does synapsis occur in meiosis? If so, during which stage?

_____

?   Can a haploid cell undergo mitosis? Explain

_____

?   Can a haploid cell undergo meiosis? Explain.

_____

?   Is the cell haploid or diploid at the beginning of Prophase II? Explain.

_____

MEIOSIS AND MENDEL'S PRINCIPLES OF INHERITANCE

      Gregor Mendel (1822-1884) was an Austrian monk interested in understanding the laws that govern inheritance. Working with a number of hereditary characteristics in the common garden pea (*Pisum sativum*), Mendel published a paper in 1866 called "Experiments in Plant Hybridization." In this paper, Mendel formulated two principles that were subsequently found to explain inheritance in many organisms. The process of meiosis was later found to be the basis for these two principles (during Mendel's time meiosis was completely unknown).

One of the genes that Mendel studied determines plant height in peas. This gene occurs as two alternate forms or alleles: an allele for tall plants and an allele for dwarf plants. When Mendel crossed true-breeding (**homozygous**) tall plants with true-breeding dwarf plants, all of the first generation (**F$_1$**) were tall. If the F$_1$ generation was allowed to interbreed, tall and dwarf plants appeared in the next generation (**F$_2$**) in the ratio of 3:1. If each of the F$_2$ plants self-fertilized, all of the dwarf plants produced dwarf offspring, while one-third of the tall plants produced tall offspring, and the other two-thirds produced both tall and dwarf offspring, again in the ratio of 3:1. These results are summarized in Figure 1.3.

Principle of Segregation

Mendel's Principle of Segregation in modern terms states that the alleles of a gene are separated during meiosis, with the result that only one allele of each gene is present in cells produced through meiosis. Specifically, segregation of alleles occurs during anaphase I of meiosis[*], since it is during that stage that homologous chromosomes are separated. For example, the F$_1$ tall pea plant possesses two different alleles (a **heterozygous** condition): the dwarf allele on one homologue of the pair bearing the gene for height, and the tall allele on the other homologue. In this case, the tall allele is **dominant** over (masks the expression of) the dwarf allele, and thus the F$_1$ phenotype is tall, even though the F$_1$ genotype is heterozygous (Tt) for this gene. When these plants undergo meiosis to form haploid cells, the two homologous chromosomes separate; one-half of the cells receive a dwarf allele (t), and the other half receive a tall allele (T). During fertilization, random recombination of the gametes derived from these haploid cells produces the phenotypic ratio of 3 tall to 1 dwarf and the genotypic ratio of 1TT : 2Tt : 1tt of the F$_2$ generation. Segregation and random recombination also explain the results obtained in the F$_3$ generation.

The law of segregation is sometimes applied at the chromosome level, describing the separation of homologous chromosomes. At the genome level, segragation may describe the separation of full sets of genome into distinct nuclei. In this lab we mainly focus on Mendel's principle of segregation at the allele level during meiosis.

Simulating the Principle of Segregation with a Monohybrid Cross

You will now use the poppet bead chromosome models to see how the behavior of chromosomes in meiosis explains this principle.

Begin with a cell from one of the F$_1$ heterozygous individuals (Tt) shown in Figure 1.3.

❑ Prepare the homologous chromosomes as they would appear in interphase <u>prior</u> to DNA replication in the Tt, F$_1$ parent cell. Use the color of the chromosomes you make to show their parental origin, either the tall or dwarf parent from the P generation. Select a specific bead as the gene for this characteristic, and designate alleles by attaching special labels on which you have written either "**T**" or "**t**".

❑ Simulate chromatid replication by snapping on another strand of beads (with the allele designated).

❑ Simulate meiosis to produce four haploid cells. In higher plants, gametes are derived from these haploid cells by mitosis.

---

[*]If a pair of alleles was involved in a crossover, segregation takes place during the second division of meiosis. You will consider this in detail in the next chapter.

P		Tall (TT)	×	Dwarf (tt)		

F₁			Tall (Tt)			

F₂	Tall (TT)	Tall (Tt)		Tall (Tt)		Dwarf (tt)

F₃	Tall (TT)	3 Tall (1 TT : 2 Tt)	Dwarf (tt)	3 Tall (1 TT : 2 Tt)	Dwarf (tt)	Dwarf (tt)

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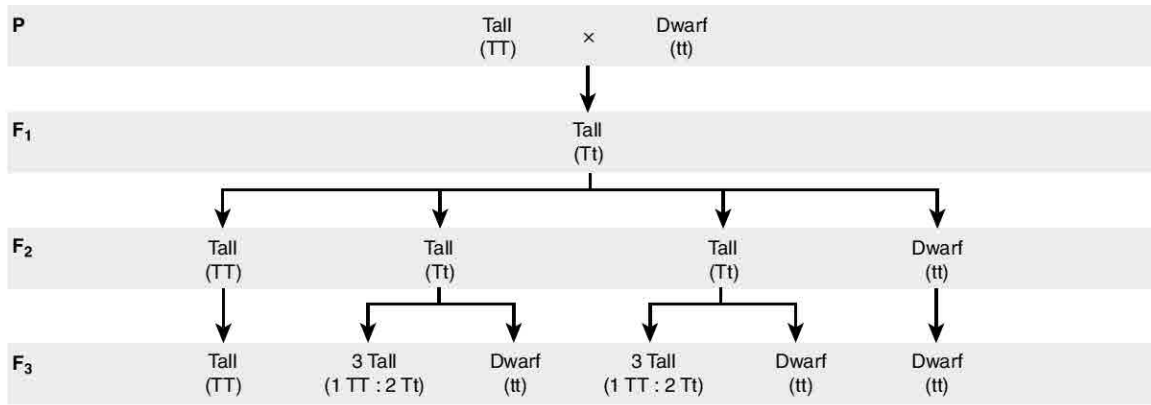
Figure 1.3. Results from Mendel's experiments with tall and dwarf varieties of peas (T = allele for tall condition, t = allele for dwarf condition).

The Punnet square is a useful device for determining the genotypes expected from a particular genetic cross. In constructing and using the Punnet square, you mimic the events of meiosis and fertilization. There are two steps in the use of the square: first, determining what kinds of gametes the parents can produce; second, combining gametes to get the offspring from the cross.

Determining Gametes - In constructing a Punnet square, you first determine what possible gametes can be produced given the genotypes of the parents. For example, in the cross Tt x Tt, half of the gametes each parent will produce will have the allele T, the other half the allele t. This is a reflection of the principle of segregation and the separation of homologous chromosomes (and the alleles they bear) during Anaphase I. These gametes head the rows and columns making up the square; the rows for gametes from the one sex, the columns for gametes of the other sex.

Combining Gametes - Now you simply fill in cells of the square by combining gametes: female T gamete + male T gamete to get one TT individual, etc. Totaling up the four squares gives 1 TT, 1 Tt, 1 tT, and 1 tt, or 1TT :2Tt :1 tt. This process of combining gametes in the Punnet square assumes that the populations of male and female gametes are combining randomly to produce the next generation.

Female gametes

	T	t
T		
t		

Male gametes
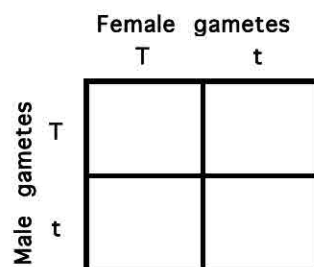
❏   Fill in the genotypes for the Punnet square shown above. Are the genotypes and phenotypes of the F₂ progeny the same as shown in Figure 1.3?

Principle of Independent Assortment

In his experiments, Mendel also performed a **dihybrid cross**; that is, he crossed individuals differing in two hereditary characteristics. For example, he crossed plants grown from round, yellow seeds with those from wrinkled, green seeds. The parent plants were true-breeding, or homozygous, for the two genes for seed coat texture and seed color. Since the F_1 individuals produced seeds that were all phenotypically round and yellow, Mendel concluded that the round allele was dominant over the wrinkled allele, and that the yellow allele was dominant over the green allele. Mendel allowed the F_1 plants to interbreed and observed the following phenotypic ratios among the F_2 seeds:

>9 round yellow
>3 round green
>3 wrinkled yellow
>1 wrinkled green

All of the other dihybrid crosses Mendel performed also resulted in a 9:3:3:1 phenotypic ratio in the F_2 generation. From these data, Mendel formulated his Principle of Independent Assortment, which, in modern terms, states that alleles of different genes are assorted independently into haploid cells during meiosis. Again, a clear understanding of meiosis explains the basis of this principle as well as the results Mendel obtained. This principle applies to physically unlinked genes, that is, genes on chromosomes that are not homologous. See details on linkage in the section on genetic concepts.

Mendel's second principle is based on the manner in which homologous pairs line up on the spindle's equator during metaphase I of meiosis. The genes determining seed texture and color in peas are unlinked (not on homologous chromosomes). For a heterozygous individual (RrYy), there are two possible arrangements of the maternal and paternal members of these two sets of homologous chromosomes. Since each arrangement is equally probable, four types of haploid cells will be formed in equal numbers (see Figure 1.4). Random fertilization with the gametes derived from these four cell types will produce the genotypic and phenotypic ratios Mendel obtained.

Simulating The Principle of Independent Assortment with a Dihybrid Cross

You will now use the poppet beads to demonstrate how meiosis explains the principle of independent assortment. Begin with a cell from one of the doubly heterozygous (RrYy) F_1 individuals.

❑ Prepare the homologous chromosomes as they would appear during interphase prior to DNA replication. Assume that the two genes are <u>unlinked</u> (on nonhomologous chromosomes). Designate the beads representing the alleles for each gene with special labels on which you have written either "**R**" or "**r**" and "**Y**" or "**y**".

❑ As before, simulate chromosome duplication by snapping on additional strands of beads (appropriately labeled).

❑ Simulate meiosis to produce haploid cells. **Note**: During Metaphase I, consider all possible arrangements of the two pairs of homologous chromosomes and determine the types of haploid cells that each arrangement would produce (use Figure 1.4 as a guide). Recall that in higher plants spores are made by meiosis and gametes are derived by mitosis from plants that grow from these spores.

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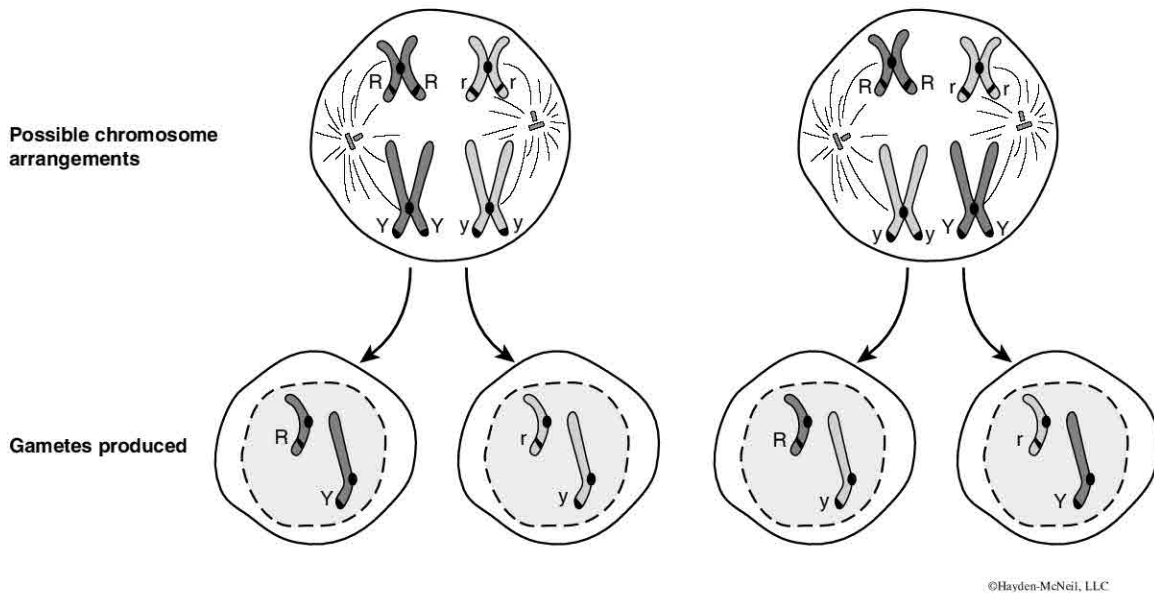
Figure 1.4. Two possible arrangements of two pairs of homologous chromosomes at metaphase I and the resulting four gametes (or spores in plants) for an individual with the genotype RrYy (R = allele for round seeds, r = allele for wrinkled seeds; Y = allele for yellow seeds, y = allele for green seeds). Stippled chromosomes are from the round, yellow parent; clear chromosomes are from the wrinkled, green parent.

As before, the first step in constructing a Punnet square involves determining what gametes the parents can produce. If the cross involves unlinked genes, you must take the principle of independent assortment into account in doing this. For example, in a cross of individuals heterozygous for two unlinked genes, four types of gametes are possible for each parent. A convenient way to determine what those gametes are is to combine the first allele for gene one with the first allele for gene two and then to combine the first allele for gene one with the second allele for gene two. This process would be repeated for the second allele of gene one to obtain the four unique gametes for each parent.

This method of determining the genotypes of gametes is based on the independent assortment of the pairs of homologous chromosomes during Metaphase I of meiosis. Because there are four different ways to combine two pairs of homologous chromosomes, when each homologue bears a different allele, there will be four different kinds of gametes and each will occur in equal frequency (about 25% of the total of all gametes). Each gamete's genotype is now used to head a row or column of the Punnet square for this cross, as shown in Figure 1.5.

RrYy

1) RY
2) Ry
3) rY
4) ry
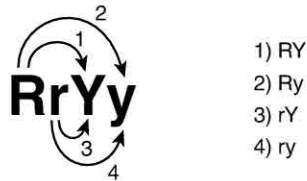
As before, combining rows and columns in the Punnet square to determine the genotypes expected from the cross assumes that male and female gametes are combining randomly to produce the next generation.

?   During which meiotic stage does segregation of the alleles from the two different parents occur?

?   During which meiotic stage does independent assortment of genes on nonhomologous chromosomes (unlinked genes) occur?

<div align="center">

Gametes

	RY	**Ry**	**rY**	**ry**
RY	RRYY	RRYy	RrYY	RrYy
Ry	RRYy	RRyy	RrYy	Rryy
rY	RrYY	RrYy	rrYY	rrYy
ry	RrYy	Rryy	rrYy	rryy

Gametes (left side)

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</div>

Genotype ratios	Phenotype ratios
1 RRYY	
2 RrYY	
4 RrYy	9 Round yellow (R_Y_)
2 RRYy	
1 RRyy	
2 Rryy	3 Round green (R_yy)
1 rrYY	
2 rrYy	3 Wrinkled yellow (rrY_)
1 rryy	1 Wrinkled green (rryy)

Figure 1.5. Punnet square and the genotypic and phenotypic results from a cross between two F₁ (RrYy) individuals. Note: Figure 1.4 shows the four types of gametes produced due to independent assortment of the two pairs of homologous chromosomes bearing the genes involved in the cross.

POST-LAB WEB ASSIGNMENT

To test your understanding of this laboratory, complete the associated web activities located in the lab section of the course web site.

URL = http://biog-1101-1104.bio.cornell.edu/BioG1101_1104/tutorials/cell_division.html

You will encounter questions similar to these on lab midterms and the final practical examination.

REFERENCES AND SUGGESTED READINGS

Campbell, N. A., Reece, J. B., Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., et al. (2008). *Biology* (8th ed.). San Francisco, CA: Benjamin Cummings.

Corcos, A. F., & Monaghan, F. V. (1993). *Gregor Mendel's experiments on plant hybrids; a guided study*. New Brunswick, NJ: Rutgers University Press.

Hawk, J. A., & Crowder, L. V. (1970). Demonstrating mitosis and meiosis. *The American Biology Teacher, 38*(2), 105-111.

Peddler, I. J., & Wynne, E. G. (1972). *Genetics: A basic guide*. New York: Northon Co.

CELL DIVISION-GENETIC CONSEQUENCES WORKSHEET

Name: _____   **Lab instructor:** _____

1.   A litter of small-sized rabbits with white fur is produced by crossing a small-sized rabbit with white fur with a large-sized rabbit with brown fur. Assume that both parents represent true breeding lines, and that there are two genes involved which are located on different chromosomes.

a)   Assign symbols for the alleles for each gene involved and list all the possible phenotypes and their matching genotypes that can result from all possible allelic combinations for each gene.

b)   Identify the genotypes of the parents that were crossed and genotype(s) of their F_1 offspring.

Parents: Offspring:

c)   Two rabbits from this new litter (the F_1 generation) are mated. Identify the genotype(s) involved in the new F_1 X F_1 cross being done.

d)   Show a Punnet square of this cross.

e)   What proportion of the offspring will be large-sized rabbits with white fur?

CHAPTER 2 – A STUDY OF THE GENETICS OF *Sordaria fimicola*

SYNOPSIS

In this laboratory you will need to apply an understanding of meiosis and Mendel's two principles of inheritance in a study of the genetics of spore color determination in the fungus *Sordaria fimicola*. You will use tetrad analysis of the interstrain asci resulting from a cross between the gray spore and tan spore mutant strains of this fungus to collect data. Analysis and transformation of class data during Day Two will allow you to determine if the tan spore and gray spore genes are linked or genetically unlinked and to map the location of each gene on its chromosome.

LEARNING OBJECTIVES

Conceptual

1.   Describe the life cycle of *Sordaria fimicola* and the relationship of meiosis to spore production.

2.   Explain how more than one gene can be involved in determining spore coloration in *Sordaria*.

3.   Explain what a crossover is, when it occurs during meiosis, and its genetic consequences.

4.   Explain how tetrad analysis of *Sordaria* asci allows one to detect when a crossover has occurred.

5.   Explain the specific predictions that the principle of independent assortment makes for the gray spore * tan spore cross, if the two genes are genetically linked.

6.   Explain the specific predictions that the principle of independent assortment makes for the gray spore * tan spore cross, if the two genes are genetically unlinked.

7.   Understand how to apply the principle of independent assortment in analyzing the wild type x mutant crosses in order to map the tan and gray spore genes on their chromosomes, and explain what is meant by first and second division independent assortment.

8.   Understand the effect of multiple crossing over on the observed frequency of parental ditype, nonparental ditype, and tetratype asci.

9.   Define the terms recombinant and non-recombinant ascospore.

10.   Define map unit and describe its relationship to the frequency of crossing over for a gene.

11.   Differentiate between a genetic linkage map and a physical map of a chromosome.

12.   Be able to express genetic map distances in ceniMorgan (cM) map units.

Methodological

1.   Properly squash perithecia for tetrad analysis of their asci.

2.   Identify the four spore coloration phenotypes.

3.   Classify asci as parental ditype, nonparental ditype, and tetratype.

4.   Be able to draw synapsed chromosome pairs with crossovers shown to produce a given tetratype ascus.

5. Be able to determine which gene is affected by a crossover in the mutant x mutant cross by examining the arrangement of spore genotypes within the ascus.

6. Gain experience with statistical testing using the chi square statistical test.

READING ASSIGNMENT

READINGS ASSIGNMENTS (should be done <u>before</u> lab period)

For Day 1: In the lab text: p. 32-36

For Day 2: In the lab text: p. 36 - up to the worksheet. Also read the χ^2 test section of Appendix 3, Statistical Reference.

Bring a calculator to lab both weeks.

This chapter requires that you use the χ^2 test to analyze data included in both the worksheet and research summary associated with this study. You should study the χ^2 tutorial in the *Stat–Tutor* area of the BioG 1101–1104 Web site. You can access this site at URL: "http://biog-1101-1104.bio.cornell.edu", using any computer with internet access.

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

Day 1, July 13

1. What is a tetrad, an ascus, an ascospore?

2. What are two main uses of tetrad analysis in studies of the genetics of fungi?

3. *Sordaria* has a haploid number of 7 (*n* = 7). How many chromosomes are present in an ascospore of this species? How many pairs of homologous chromosomes are present in an ascospore of this species?

4. Which stage(s) of the *Sordaria* life cycle is(are) diploid?

5. In doing tetrad analysis of *Sordaria fimicola*, the phenotypes of what structures are studied?

6. Consider the following arrangements of spores in an ascus. Which of these could result from a zygote formed from an interstrain mating between the wild type and tan spore strain?

    A. 2 tan:2 black:2 tan:2 black
    B. 4 tan:4 black
    C. 2 black:4 tan:2 black
    D. 8 tan

    How could arrangement D have occurred?

7. How many recombinant ascospores are present in an ascus with a 4 black:4 tan spore arrangement?

8. How many recombinant ascospores are present in an ascus with a 2 black:2 gray:2 black:2 gray spore arrangement?

Day 2, July 14

1. What is the difference between a parental ditype (PD) and a nonparental ditype (NPD) ascus?

2. You inoculate an agar plate with mycelia from the tan spore and gray spore strain of *Sordaria fimicola*. Some asci with the following spore arrangements are produced:

    2 gray:2 tan:2 gray:2 tan and 2 colorless:4 black:2 colorless

    Are these ditype or tetratype asci? Explain your answer.

3. From the plate set up in 2., you isolate an ascus with 8 tan spores. How could this ascus be produced? How many recombinant ascospores are present in this ascus?

4. For the plate set up in 2., how many recombinant ascospores are present in an ascus with a 4 tan:4 gray spore arrangement? How could this ascus be produced?

5. For the plate set up in 2., how many recombinant ascospores are present in an ascus with a 2 gray:2 black:2 tan:2 colorless spore arrangement? How could this ascus be produced?

6. In studying a particular *Sordaria* cross, you examine a sample of 500 asci. How many complete meiotic events (meiosis I and II) does this sample represent?

7. What is the expected proportion of PD and NPD asci if the tan spore and gray spore genes assort independently?

8. What is the expected proportion of PD and NPD asci if the tan spore and gray spore genes are genetically linked?

Jon C. Glase

Revised June 2010
Mark A. Sarvary

INTRODUCTION

The Genetics Of Fungi

Because of the existence of dominance-recessive relationships among alleles, one of the problems inherent in genetic studies of diploid organisms, like Mendel's pea plants, is the difficulty of inferring genotypes from phenotypes. For example, in peas, homozygous dominant individuals (TT) and heterozygous individuals (Tt) are phenotypically the same, both tall. In contrast, haploid organisms have only one chromosome for each of the chromosome types, so they have only one allele for each gene. This simplifies the interpretation of genetic crosses considerably because each allele is expressed in the phenotype. For this reason, and another discussed shortly, geneticists have long favored haploid organisms like the fungi as subjects for their studies.

Early genetic studies with the fruit fly, *Drosophila melanogaster*, suggested that a mechanism must exist to allow exchange of genetic material between homologous chromosomes. As was discussed earlier, microscopic studies of meiosis show that this exchange, called **crossing over**, takes place during prophase I when homologous chromosomes are in **synapsis**. During crossing over, breakage-refusion points called **chiasmata** develop between synapsed homologous chromosomes. These chiasmata result from pieces of the two homologues being switched in an equal and reciprocal fashion. Crossing over combines genetic material that had previously been on separate homologues and produces individuals with increased genetic variation. Geneticists also came to realize that crossing over could be used as an important tool for learning more about the location of genes on chromosomes. They reasoned that if chiasmata can form at any point between two homologous chromosomes, then the frequency of crossing over in the region between two different genes on a chromosome should vary directly with the physical distance between the genes. When this hypothesis was confirmed it was possible to begin mapping the positions of genes on chromosomes.

In genetic studies of animals, a **cross**, or mating, is made between parents whose genotypes may be partially known. These parents contain gametes that have resulted from many meiotic divisions within their gamete-producing structures. Each meiotic division produces four haploid nuclei collectively called a **tetrad**. In most organisms the products of each meiotic division are not kept ordered but become part of a "pool" of meiotic products (gametes). The mating activities of the parents combine these meiotic products in a random fashion to produce the next generation. Thus, in most organisms, it is impossible to examine the assortment of alleles in an individual meiotic division. However, such a genetic description of an individual meiotic division would be particularly advantageous in studying the occurrence and frequency of crossing over.

The fungal life cycle is different from that of animals in that meiosis makes spores, not gametes (Fig. 2.1). In certain fungi such as the pink bread mold, *Neurospora crassa,* and *Sordaria fimicola* (the organism you will study during the next two days), meiosis occurs within a structure called an **ascus**, which isolates each tetrad. The four products of meiosis occur within the ascus in the spatial arrangement in which they arose during meiosis. With these organisms, a special type of genetic analysis called **tetrad analysis** can be used. In tetrad analysis, the genetic make-up of each cell of a tetrad can be studied with respect to a particular trait, and this information can be related to the meiotic division that produced the tetrad. Tetrad analysis makes it possible to determine when crossing over has occurred, and to use this information to determine if genes are linked and to map the locations of genes on chromosomes.

Sordaria Life Cycle (see Figure 2.1)

Fertilization consists of two separate, sequential events. First, fusion of the contents of two cells, called **plasmogamy,** forms one cell with two haploid nuclei. Second, fusion of the haploid nuclei, called **karyogamy**, forms a zygote with a diploid nucleus. In most organisms, karyogamy immediately follows plasmogamy. In the fungi, cells with two nuclei (called **dikaryotic cells**) remain for extended periods of time. Only at a later point when karyogamy occurs is a diploid zygote formed. In all the fungi, the zygote is the only stage in its life cycle to have a diploid nucleus.
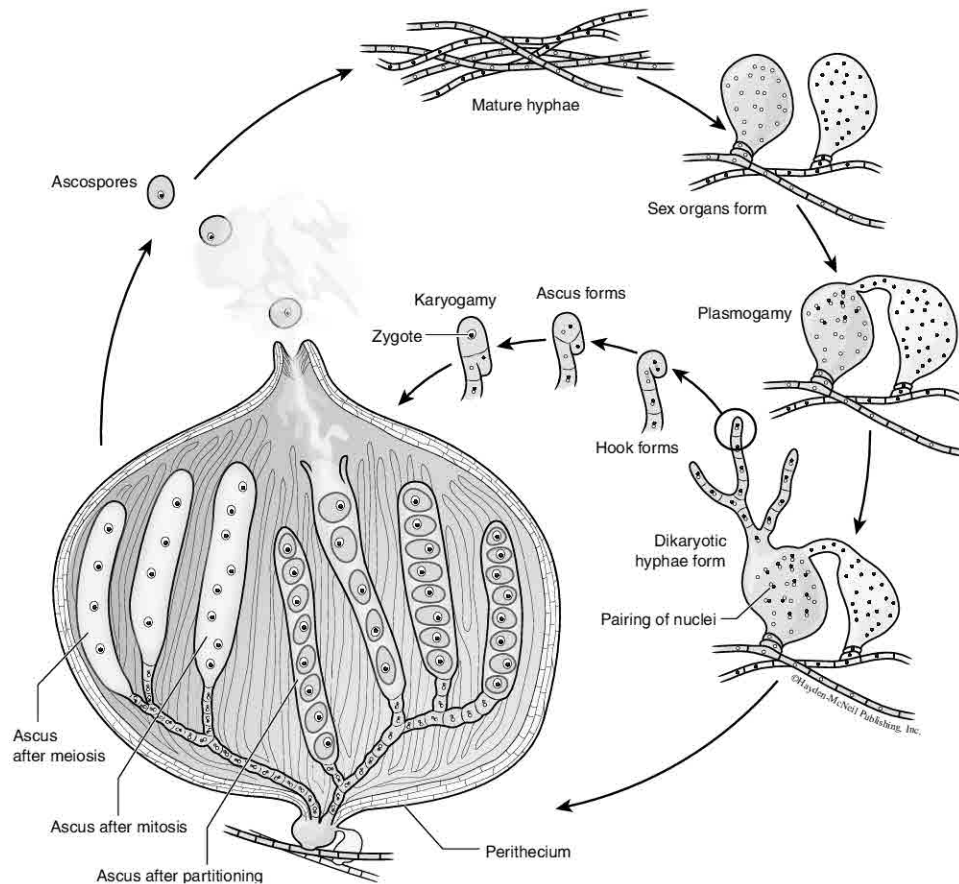
Figure 2.1. The life cycle of the fungus *Sordaria fimicola* (Rushforth 1976).

In *Sordaria fimicola*, the multicellular fungal body is composed of haploid cells arranged in long filaments called **hyphae**; the whole network of filaments is called a **mycelium**. If conditions are right, the fungus forms sex organs containing numerous haploid nuclei. Nuclei then migrate from one organ to the other where they pair up. Budding off forms dikaryotic hyphae. Karyogamy occurs within an ascus primordium to form a diploid zygote. Each zygote immediately undergoes meiosis, producing four haploid nuclei which each divide once, mitotically, yielding a total of <u>eight</u> haploid cells. Each cell develops a resistant cell wall and is called an **ascospore**. All of these events occur within an elongate sac called an **ascus**, so that at maturity an ascus contains eight ascospores arranged in a precise manner. Clusters of asci develop within a special dispersal structure called a **perithecium**. As the spores within each ascus mature, the ascus tip ruptures, and hydrostatic pressure within the ascus propels the spores out of the perithecium. The entire life cycle from spore germination to production of new spores in perithecia takes about 10 days.

One important feature of ascus-forming fungi is their special spore formation process. The meiotic and mitotic divisions producing eight ascospores from one zygote are subject to the physical limitations imposed by the shape of the ascus. The long, thin ascus causes both meiotic spindles and the mitotic spindles to be lined up during spore formation. As a result, the positioning of the ascospores directly reflects the arrangement of homologous chromosome pairs during metaphase I of meiosis. Also, since ascospores are haploid, all alleles are phenotypically expressed. The direct visual determination of genotype and phenotype in their haploid cells, and their short generation time, are two additional reasons for the extensive use of these fungi in genetics.

The Genetic Determination of Spore Coloration

The spore color of the normal (wild type) *Sordaria* is black. Several different genes are involved in producing this phenotype and each gene has two possible allelic forms. We will be concerned with two spore color genes. The **gray spore gene** has two allelic forms: the wild type allele (g^+) and a mutant allele (g). The **tan spore gene** also has two forms: a wild type allele (t^+) and a mutant allele (t). Normal black spores are produced only if both wild type alleles are present at the loci of both genes. Thus, black ascospores have the genotype g^+t^+ (remember, spores are haploid). Those with the genotype g t^+ are gray, while g^+t ascospores are tan. Ascospores that are g t show a cumulative effect of the two mutations and are colorless.

The black coloration of wild type *Sordaria* spores is due to the production of the pigment melanin and its deposition in the cell walls. As with most complex biochemicals, melanin is produced within the cell by a series of coupled reactions called a **biosynthetic pathway**. You need to consider this pathway in order to better understand the inheritance of spore coloration in *Sordaria*.
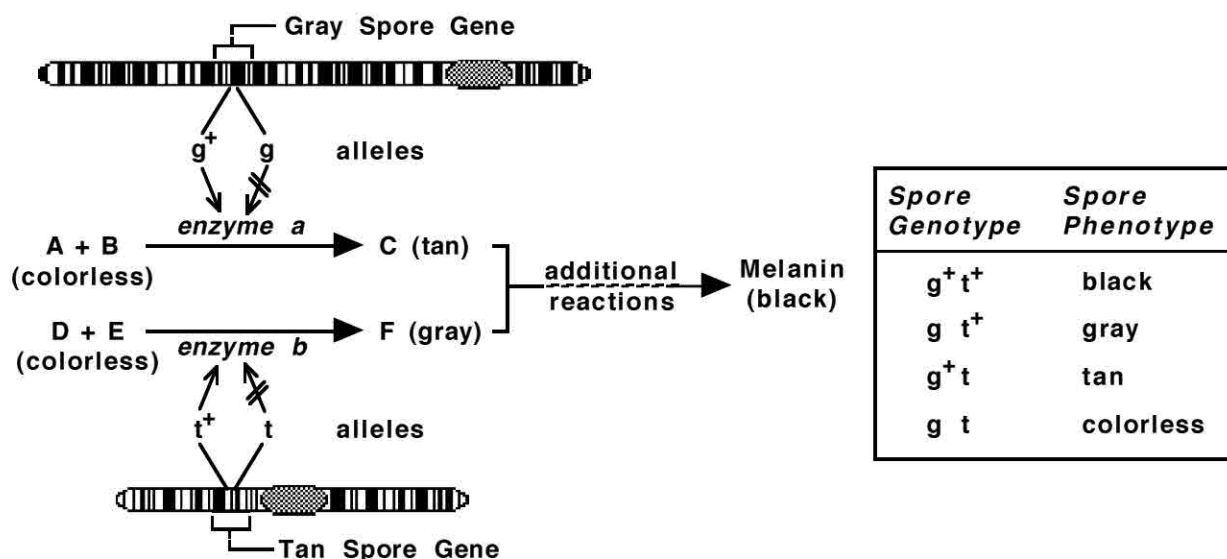
Figure 2.2. Hypothetical biosynthetic pathway for the black pigment melanin showing the involvement of particular genes via their production of enzymes for reaction steps in the pathway. Letters A-F designate intermediate compounds in the pathway. Note: placement of the two genes on separate, non-homologous chromosomes is arbitrary.

Figure 2.2 shows a hypothetical biosynthetic pathway for melanin production. In this pathway each reaction is catalyzed by a different enzyme. Suppose that the gray spore gene codes for *enzyme a*, and the tan spore gene for *enzyme b*. The wild type alleles for both genes (**g^+** and **t^+**) produce the normal

enzymes which allow melanin biosynthesis and the formation of black ascospores. However, mutant alleles for either gene (**g** and **t**) do not produce the normal enzymes and block the pathway. For example, if the mutant allele of the gray spore gene (**g**) is present, *enzyme a* will not be produced. This blocks production of substance C, and substance F accumulates in the ascospore cell walls, causing them to appear gray. If, alternatively, the mutant allele of the tan spore gene (**t**) is present, *enzyme b* will be absent, and substance F will not be produced. This causes substance C to accumulate in the cell walls, and these spores appear tan. With both genes represented by mutant alleles (**g** and **t**), both C and F are absent, and the cell walls appear colorless. Other genes code for enzymes catalyzing other steps in the pathway. The wild type alleles for all the genes must be present within the spore if melanin is to be produced. You will gain more experience with the genetic control of biosynthetic pathways in Chapter 3.

Observing *Sordaria* Life Cycle Stages

❑	Examine plates containing colonies of the tan spore and gray spore strains of *Sordaria fimicola*. The plates were inoculated by placing small pieces of agar containing mycelia from the parent strains on to the plate.

❑	Note the fuzzy appearance on the plate surface; this is the fungal **mycelium** composed of branched filaments known as **hyphae**. Hyphae contain haploid nuclei. Because the spore color genes affect melanin production and deposition in the cell walls of the hyphae as well as the spores, you should be able to see a difference in the color of the mycelia on the two different plates.

❑	Look for many small black objects on the surface of the agar. These are the perithecia or spore-dispersal organs of the fungus. Each contains numerous asci, in various stages of development.

❑	Note a dusty coating on the lid in areas immediately above regions on the plate where perithecia are common. These are deposits of ascospores that have been ejected from perithecia.

Crossing Over in *Sordaria*

Imagine that a nutrient agar plate was inoculated with mycelia from the two *Sordaria* strains, wild type and gray spore or&

$$g^+t^+ \text{ X } g\ t^+$$

(In order to simplify the discussion and figures that follow we will omit the tan spore gene from our consideration because in this cross both strains have wild type (**t⁺**) alleles for this gene.)

On this plate, mycelia would grow together, mate, and produce numerous perithecia, each containing many asci. A zygote resulting from this cross could produce the ascospore arrangement depicted in Figure 2.3. Note that the ordering of the ascospores within the ascus reflects the arrangement of homologous chromosomes during metaphase I because of the alignment of meiotic and mitotic spindles due to the long, thin shape of the ascus.

In examining the spore arrangements within asci, you will find the six different types depicted in Figure 2.5. Two of these arose in the manner indicated in Figure 2.3; independent assortment of the chromosome bearing the gray spore gene with no crossing over. The right hand arrangements in Figure 2.5 were produced by crossing over between the centromere and the locus of the spore color gene. An example of crossing over is given in Figure 2.4. As you recall, crossing over occurs during prophase I and exchanges sections of non-sister chromatids. The spore pattern produced depends on which chromatids of the homologous chromosomes were involved in the crossover and also their orientation during metaphase I.

❏ Your Lab Instructor will project several slides showing examples of the ascus types resulting from crosses between the wild type and gray spore strains (g^+t^+ x g t^+) and the wild type and tan spore strains (g^+t^+ x g^+ t). Be sure you can identify the different spore phenotypes and consider the kind of crossovers required to produce the spore arrangements shown.
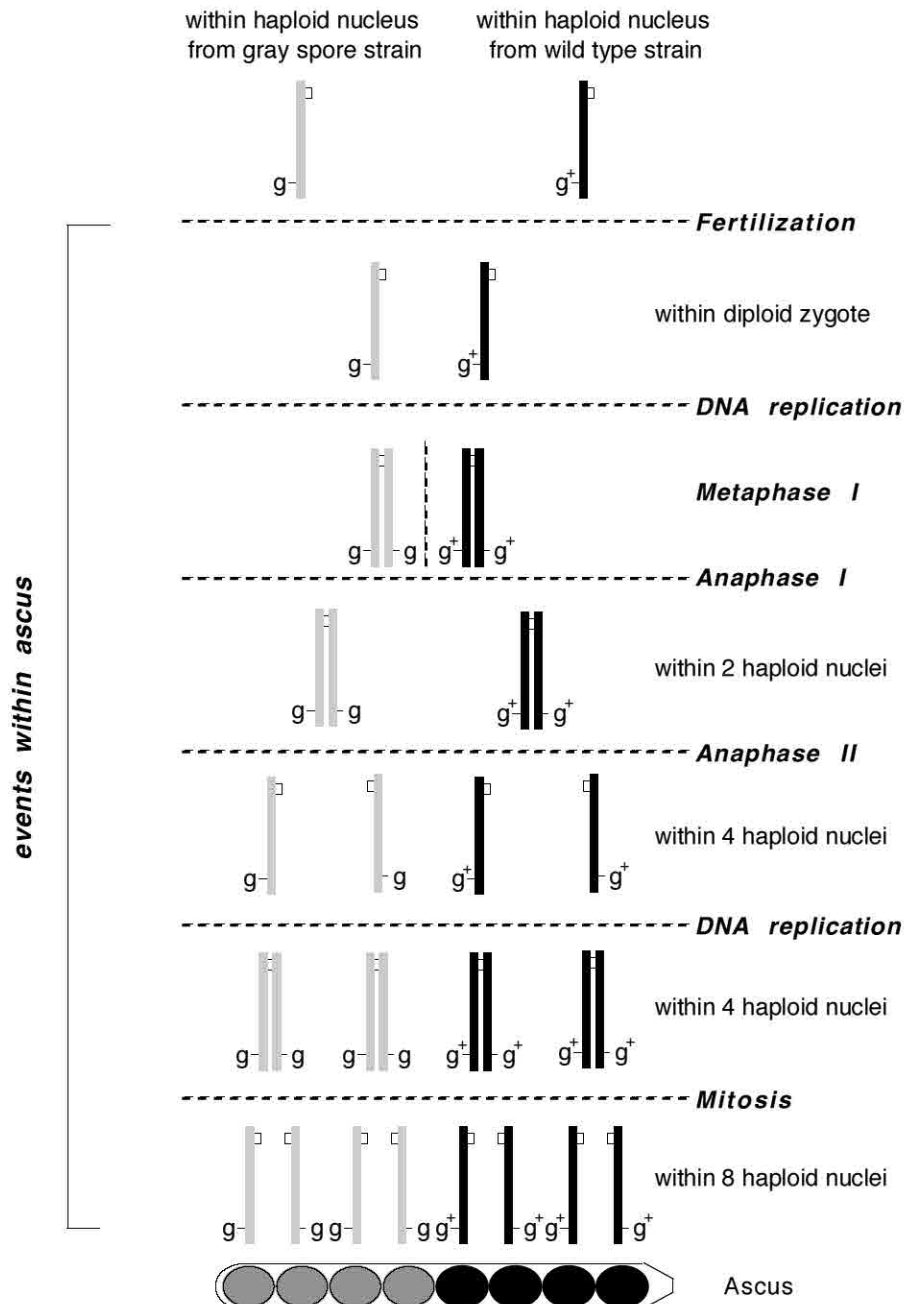
Figure 2.3. Ascospore pattern from a cross between the wild type, black spore strain and the mutant, gray spore strain. Note: because the tan spore gene is represented by wild type alleles in both strains, for simplicity it is omitted from these drawings.
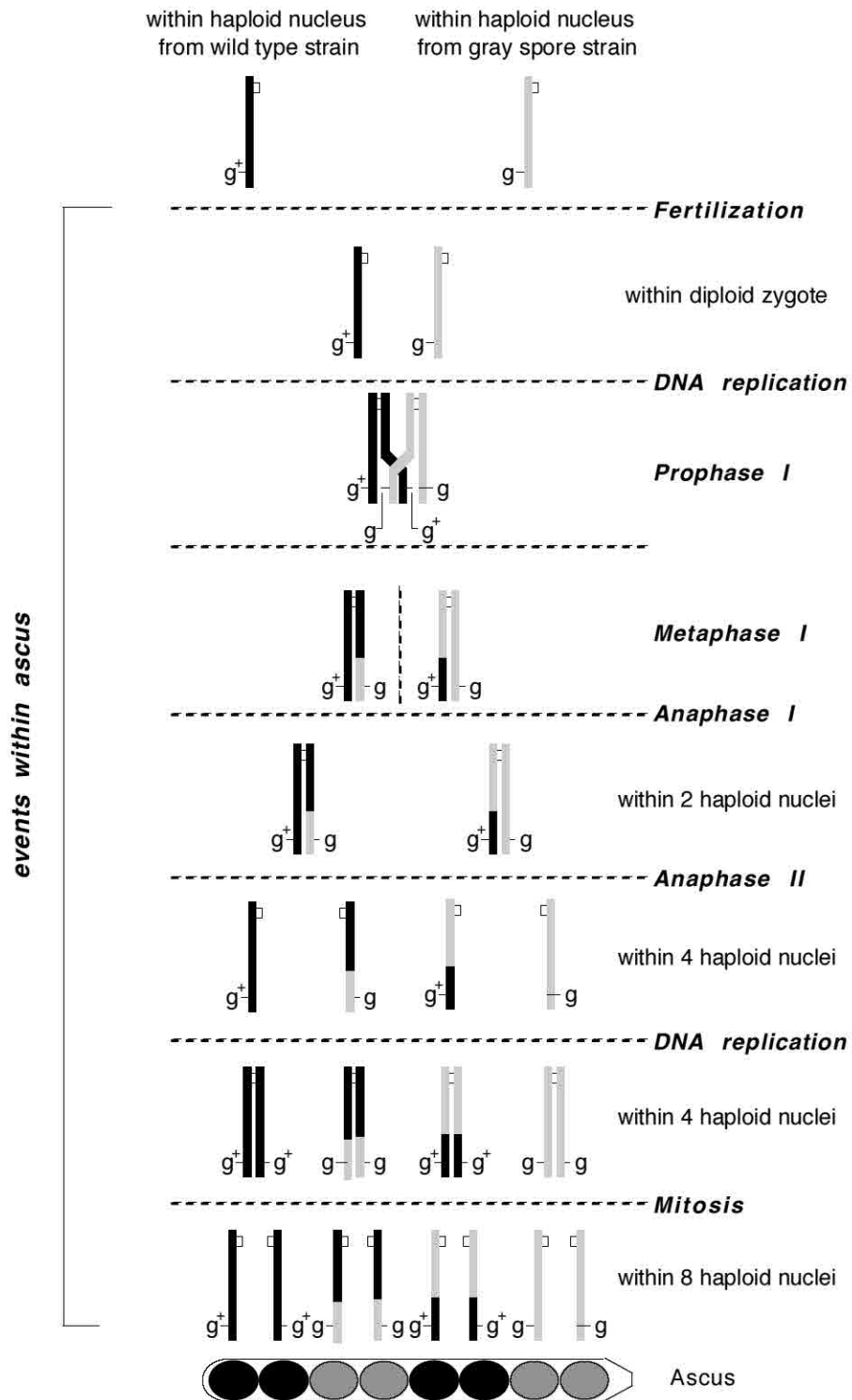
Figure 2.4. Ascus formation in *Sordaria fimicola* from a mating between the wild type, black-spored strain and the mutant, gray spore strain. A crossover during prophase I produces one of the four crossover ascus types.

Non-crossover Types   **Crossover Types**
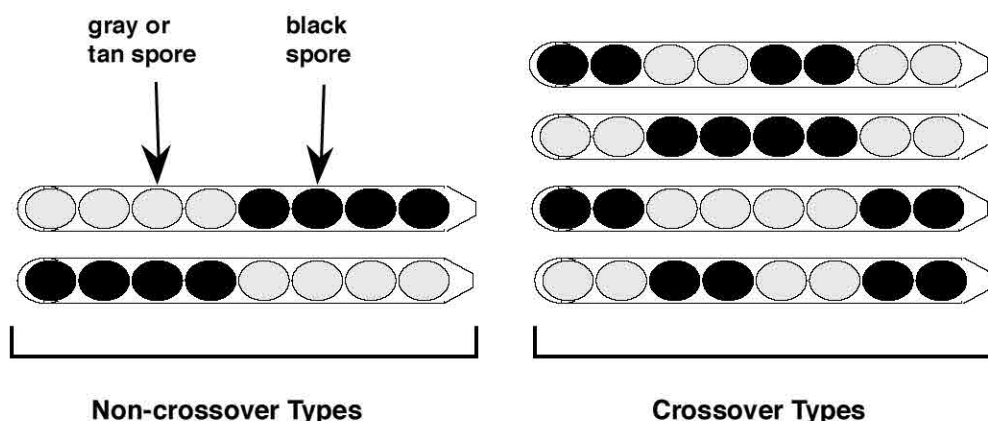
Figure 2.5. Possible ascospore arrangements produced when the mutant, gray or tan spore strain is crossed with the wild type, black spore strain.

THE GRAY SPORE ´ TAN SPORE CROSS – PREDICTIONS AND DATA COLLECTION

Two weeks ago, a cross was set up between the gray spore mutant strain and the tan spore mutant strain of *Sordaria fimicola*.

　　　　　gray spore strain　　　　　　　　*tan spore strain*
　　　　　　　g t$^+$　　　　　　X　　　　　　g$^+$t

Because the parental strains have different alleles for both genes (unlike the cross between the wild type x gray spore strain you modeled earlier), you will be able to observe the interaction of both genes in determining spore color. Recall that black spores have a wild type allele for each gene (g$^+$t$^+$) and colorless ascospores have a mutant allele for each gene (g t). (You may wish to review the information on spore color determination presented earlier in this chapter.)

How will you collect data and interpret the results from this cross in order to decide if the two genes are genetically linked? The answer to this question can be simply derived if you return to the process of meiosis and Mendel's principle of independent assortment.

According to the **Principle of Independent Assortment**, if two genes are genetically unlinked they should assort independently of each other at metaphase I or metaphase II of meiosis. Independent assortment of the tan and gray spore genes will lead to certain predictable results in the cross involving the two mutant strains. Linked genes will not assort independently, and, if this is the case, the results from the mutant-mutant cross will be quite different from those in the unlinked situation. The criterion, then, for deciding if two genes are linked or genetically unlinked is whether they show independent assortment. Consider these two hypothetical situations and examine the predictions that each makes for this cross.

Unlinked Gene Hypothesis

Consider Figure 2.7. Because pairs of homologous chromosomes line up independently of each other at metaphase I of meiosis, two possible arrangements of two pairs of homologous chromosomes (Pair 1 and Pair 2) are possible. Assume that lighter chromosomes for both pairs came from one parent, and the darker chromosomes came from the other parent. In a large number of cells undergoing meiosis, one-half of the cells will have both darker chromosomes on one side of the spindle's equator and both lighter chromosomes on the other side (Arrangement A). In the other one-half of the cells, the chromosomes will have Arrangement B.
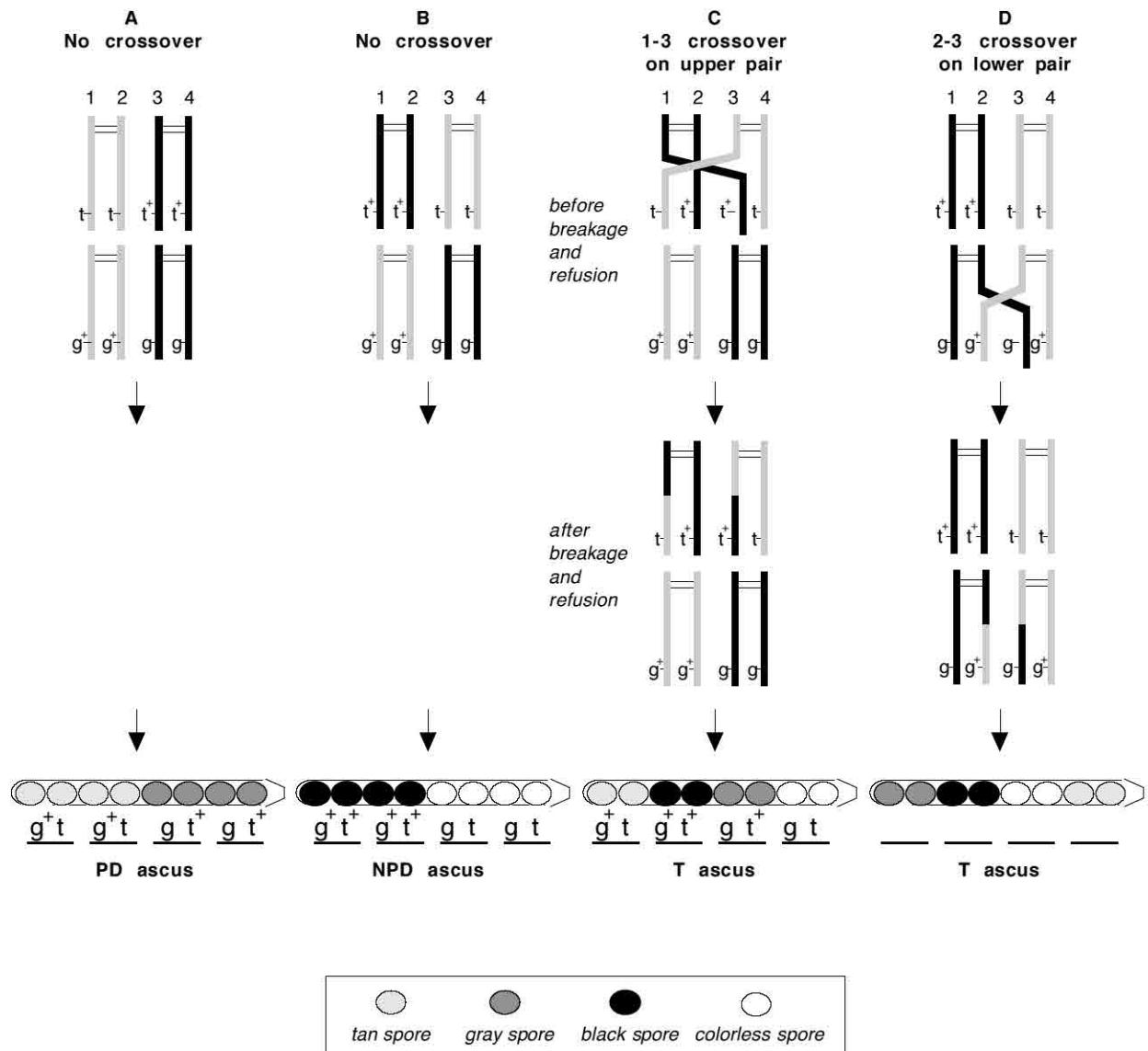
Figure 2.6. Predicted ascus types from the unlinked gene hypothesis for the tan and gray ascospore color genes. Note: Darker homologues come from the gray spore strain; lighter homologues come from the tan spore strain. A-B show independent assortment with no crossing over; C-D show examples of crossovers involving a single pair of chromosomes.
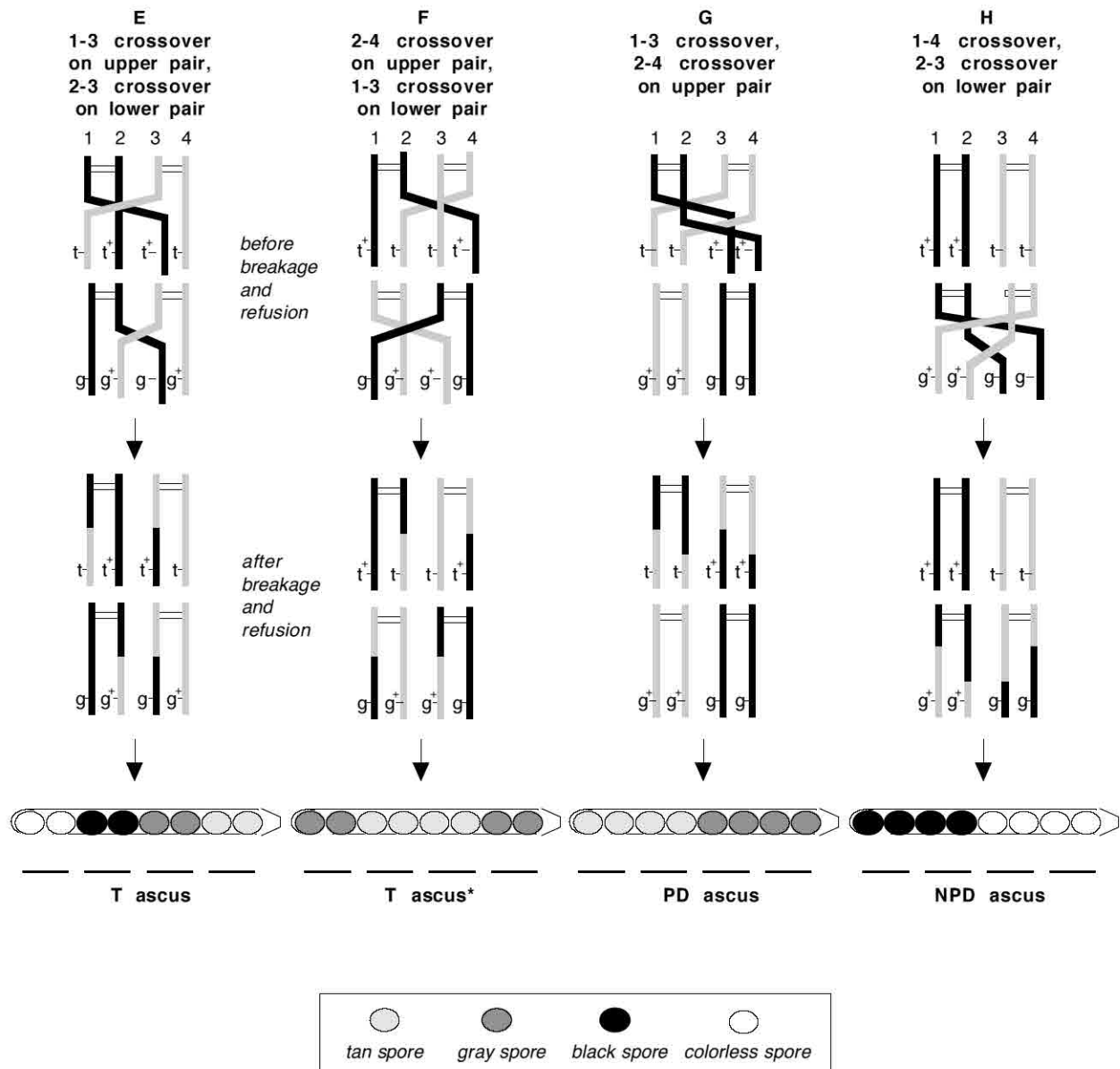
Figure 2.6 (continued). Predicted ascus types from the unlinked gene hypothesis for the tan and gray ascospore color genes. <u>Note</u>: Darker homologues come from the gray spore strain; lighter homologues come from the tan spore strain. E-F show examples of crossovers involving both pairs of chromosomes and G-H shows examples of double crossovers between one pair of homologous chromosomes.

*   Even though this ascus has only two spore color phenotypes and they are the parental phenotypes, this is still classified as a tetratype ascus because the spores are in a non-4:4 arrangement, indicating that the ascus contains recombinant (resulting from crossover) spores.
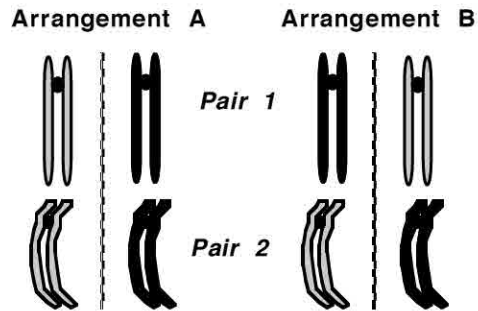
Figure 2.7. The independent assortment of two pairs of nonhomologous chromosomes (Pair 1 and 2) on the spindle's equator at metaphase I of meiosis. Darker and lighter chromosome color show parental origin.

In Figure 2.6, the upper homologous pair bears the alleles for the tan spore gene and the lower pair bears the alleles for the gray spore gene. If we assume that the lighter chromosomes for both pairs came from the tan spore parent (g^+t) then the alleles present on the lighter chromosomes would be g^+ and t. If the darker chromosomes come from the gray spore parent (g t^+), then the alleles present should be g and t^+. **A** and **B** in Figure 2.6 show the two possible arrangements of these nonhomologous chromosomes, due to independent assortment, and the resulting ascus types. Both kinds of asci shown in Figure 2.6 **A** and **B** are the type produced by a meiotic division in which <u>no</u> crossing-over occurred; only two genotypes of spores are present in each ascus and they are arranged in a 4:4 pattern. In **A**, one-half of the spores have a g^+t genotype like the tan parent; the other half have a g t^+ genotype like the gray parent. This ascus arrangement is called a **Parental Ditype (PD)** ascus– <u>ditype</u> because only <u>two</u> genotypes are represented in the ascus and <u>parental</u> because both genotypes are like those of the parents. In **B** there are also only two genotypes present (g^+t^+ and g t) but neither is like the parents' genotypes. This ascus arrangement is called a **Non-Parental Ditype (NPD)** ascus– <u>ditype</u>, again, because only two genotypes are represented and <u>non-parental</u> because both genotypes differ from the parents.

Situations **C** and **D** of Figure 2.6 show two examples of single crossovers occurring between the centromere and the tan locus (**C**), and the centromere and the gray locus (**D**). Recall that there are four different ways a crossover can occur between the non-sister chromatids for each pair of homologous chromosomes. In all such cases, an ascus results which contains spores of all <u>four</u> genotypes, arranged in a 2:2:2:2 sequence. It is also possible for both chromosomes to be involved in single crossovers. Some of these will produce asci with four spore genotypes in a 2:2:2:2 arrangement (See **E**); others (see **F**) will yield asci with only two spore genotypes, but arranged in a 2:4:2 pattern. All of these <u>non-4:4</u> <u>asci</u> are called **Tetratype (T)** asci.

Situation **G** and **H** in Figure 2.6 shows how some multiple crossovers involving the chromatids of a single chromosome type can lead to asci that are PD or NPD even though crossover did occur. We shall later discuss how the occurrence of these additional, crossover-produced PD and NPD asci will be a source of error in attempts to map the locations of these genes. However, since these types of crossovers produce equal numbers of PD and NPD asci, their existence does not change our expectations for the tan spore ´ gray spore cross. In summary, we can state the prediction of the **unlinked gene hypothesis** as follows:

If the gray spore and tan spore genes are unlinked, **then** the two genes should show independent assortment and the frequency of PD and NPD asci should be equal.

❑   In the space provided below each ascus, fill in the genotypes for the four pairs of spores for each ascus shown in Figure 2.6. Several have been done for you.

Linked Gene Hypothesis

If both spore-coloration genes are located on the same chromosome, and are within 50 map units of each other, then they are **genetically linked** and will not assort independently. However there are two ways for these two genes to behave in a genetically unlinked manner. The first occurs when these two genes are located on different, non-homologous, chromosomes. The second occurs when these two genes are on the same chromosome, **physically linked** in the same DNA molecule, but are separated by 50 map units or more. In this case there would be so much crossing over that the genes, even though physically linked, will behave in a genetically unlinked manner and will independently assort. In Figure 2.8, the right-hand homologue came from the tan spore parent (it has a t and g^+ allele) while the left-hand homologue came from the gray spore parent (it has a t^+ and g allele). If the two genes are linked and no crossing over occurs (Figure 2.8 **A**), only PD asci will result. In Figure 2.8 **B**, a crossover occurs between the tan spore gene locus and its centromere. In Figure 2.8 **C**, the crossover is between the two gene loci. Both produce T asci. **Note**: the placement of the gray and tan gene loci in this diagram is arbitrary and does not imply anything about their true locations.

Obviously, if a single chiasma can form between two chromatids, several chiasmata can also form, resulting from double, triple or higher order crossovers. Also, since there are two sets of chromatids available, it is possible for more than two chromatids to be involved in crossovers at the same time. A two-chromatid, double crossover is shown in Figure 2.8 **E**. Figure 2.8 **D** and **F** show four-chromatid double crossovers. Notice that some multiple crossovers (Figure 2.8 **E** and **F**) are not genetically detectable, because they produce PD or NPD asci. These types of multiple crossover cause us to underestimate map unit distances. Notice also that the NPD ascus produced in Figure 2.8 **F** could be interpreted as evidence for non-linkage. However, because the probability of multiple crossovers is low and because multiple crossovers produce, on average, equal numbers of PD and NPD asci, this complication does not change our expectations for the tan spore ´ gray spore cross.

In summary, if two genes are linked, we would expect mostly PD and T asci, but only a small proportion of NPD asci. We can state the prediction of the **linked gene hypothesis** as follows:

If the gray spore and tan spore genes are linked, **then** the two genes should <u>not</u> show independent assortment and the PD asci frequency should be greater than the NPD asci frequency.

❑   In the space provided below each ascus, fill in the genotypes for the four pairs of spores for each ascus shown in Figure 2.8. Several have been done for you.

Mapping Genes on Chromosomes

The exchange of genetic material between homologous chromosomes which occurs during crossing over creates a major exception to Mendel's principles. Recall that the segregation of alleles from the two parents occurs during anaphase I of meiosis, that is, during the first division of meiosis. If crossing over occurs, however, the alleles rearranged by the crossover are not segregated until anaphase II of meiosis, that is, during the second division of meiosis. Compare Figures 2.3 and 2.4 earlier in this chapter. Thus, it is said that crossing over leads to **second division independent assortment** of the alleles involved in the crossover. Gene mapping became possible when it was realized that the frequency of crossing over was related to the physical distance separating the genes involved.

In Figure 2.6 **C**, notice that only four of the eight ascospores are genetically changed due to crossover; the two tan spores with copies of chromatid 1 and the two gray spores with copies of chromatid 3. These spores are called **recombinant** ascospores because they contain chromosomes that have been changed by the crossover, and are now a combination of the parental chromosomes. The other four spores that bear chromosomes not affected by the crossover and are still like the parental chromosomes, are called **non-recombinant** ascospores.

❑ Study Figure 2.6 **D** and identify the recombinant and non-recombinant spores resulting from that crossover.
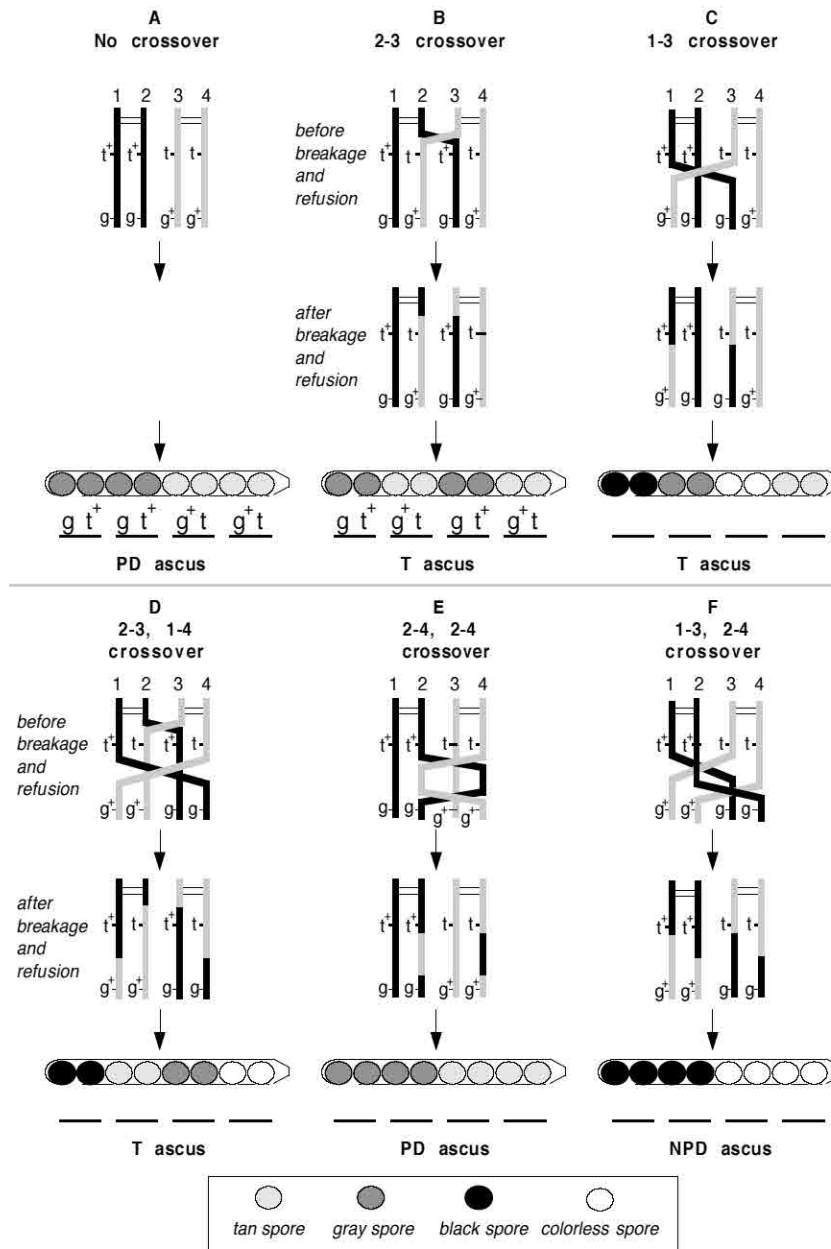
Figure 2.8. Predicted ascus types from the linked gene hypothesis for the tan and gray spore coloration genes. Darker homologue comes from the gray spored strain; lighter homologue comes from the tan spored strain.

Some thought should convince you that only crossovers between the spore color gene locus and its centromere will result in a T ascus. Crossover beyond the gene locus or on the other arm of the chromosome will **not** affect the gene under consideration. If we assume that crossing over can occur at any point along a chromosome, it is logical that the probability of a crossover occurring between a gene locus and the centromere will be proportional to the locus-centromere distance. Therefore, we can use the

frequency (proportion) of crossover-produced recombinant ascospores as a measure of the relative distance separating the gene locus and the centromere. Geneticists define a crossover **map unit** as the distance on a chromosome that produces one recombinant post-meiotic product per 100 post-meiotic products. Here, the number of map units would be equal to the number of recombinant ascospores per 100 total ascospores (both recombinant and non-recombinant).

$$\textbf{map units} = \frac{\textbf{number recombinant spores}}{\textbf{total spores (recomb. + non-recomb.)}} \times \textbf{100}$$

Given that map units express the percent recombinant spores resulting from crossovers and each single crossover produces 4 recombinant spores and 4 non-recombinant spores, the map unit distance is always one half the frequency of crossing over for the gene.

Notice that if you are using tetrad analysis to map genes in a cross involving two genes, you must differentiate between tetratype asci that result from single crossovers affecting only one gene, such as those shown in Figure 2.6 **C** and **D**, and tetratype asci that result from double crossovers affecting both genes, such as those shown in Figure 2.6 **E** and **F**. We shall return to this idea later in this study.

Using tetrad analysis, geneticists have been able to obtain genetic linkage maps of chromosomes of many organisms. These maps indicate the sequence of genes on chromosomes and the relative locations of these genes. However, because a genetic map is based on crossover frequencies, the relative distances between genes do not correspond to real, physical distances. That is, although the sequence of genes is correct, some genes may be closer together and others farther apart than genetic maps indicate. This is because some regions of chromosomes have a greater, or lesser, tendency to form crossovers than other regions. For example, the centromere seems to inhibit crossing over and genes located close to it do not crossover as much as they should based solely on their physical location.

Squashing Perithecia

Because *Sordaria* is self-fertile, some of the perithecia you will examine have resulted from intrastrain matings. Perithecia resulting from self-mating will contain asci with all tan or all gray ascospores. Since an ascus must contain at least two different phenotypes in order to detect a crossover, you will be concerned exclusively with perithecia that have resulted from interstrain matings. Perithecia containing interstrain asci (with spores of at least two different phenotypes) should be most abundant in the areas of the dish where the two strains grew together. These crosses will not be reused, so you need not use sterile technique to obtain perithecia.

❑   Each pair of students should assemble the following equipment:
    "   inoculating loop
    "   microscope slides and coverslips
    "   plastic beaker with water and eye dropper
    "   two petri dishes with the gray spore (g t$^+$) ´ tan spore (g$^+$t) cross
    "   box of Kimwipes$^®$.

❑   Each individual student should do the following:

❑   Place a drop of distilled water on a clean microscope slide.

❑   Remove the petri dish lid.

❑ Remove 10 to 15 perithecia by scraping the loop's tip back and forth over the <u>surface</u> of the agar. <u>Do not dig into the agar</u>.

❑ Place the perithecia into the drop on the slide and uniformly distribute the perithecia throughout the drop. Place a cover slip over the perithecia and water.

❑ Place a Kimwipe® over your finger (to avoid putting a fingerprint on the cover slip) and push down gently, but a little firmly, on the cover slip. If you rub the cover slip back and forth (<u>very slightly</u>) over the slide, you will spread the asci better.

❑ View the slide with the compound microscope first with the 10x objective in place.

❑ Select for study only those perithecia that contain asci with more than one phenotype. Identify the phenotypes of the spores in each ascus with the 40x objective in place. Figure 2.9B shows a properly squashed perithecium.
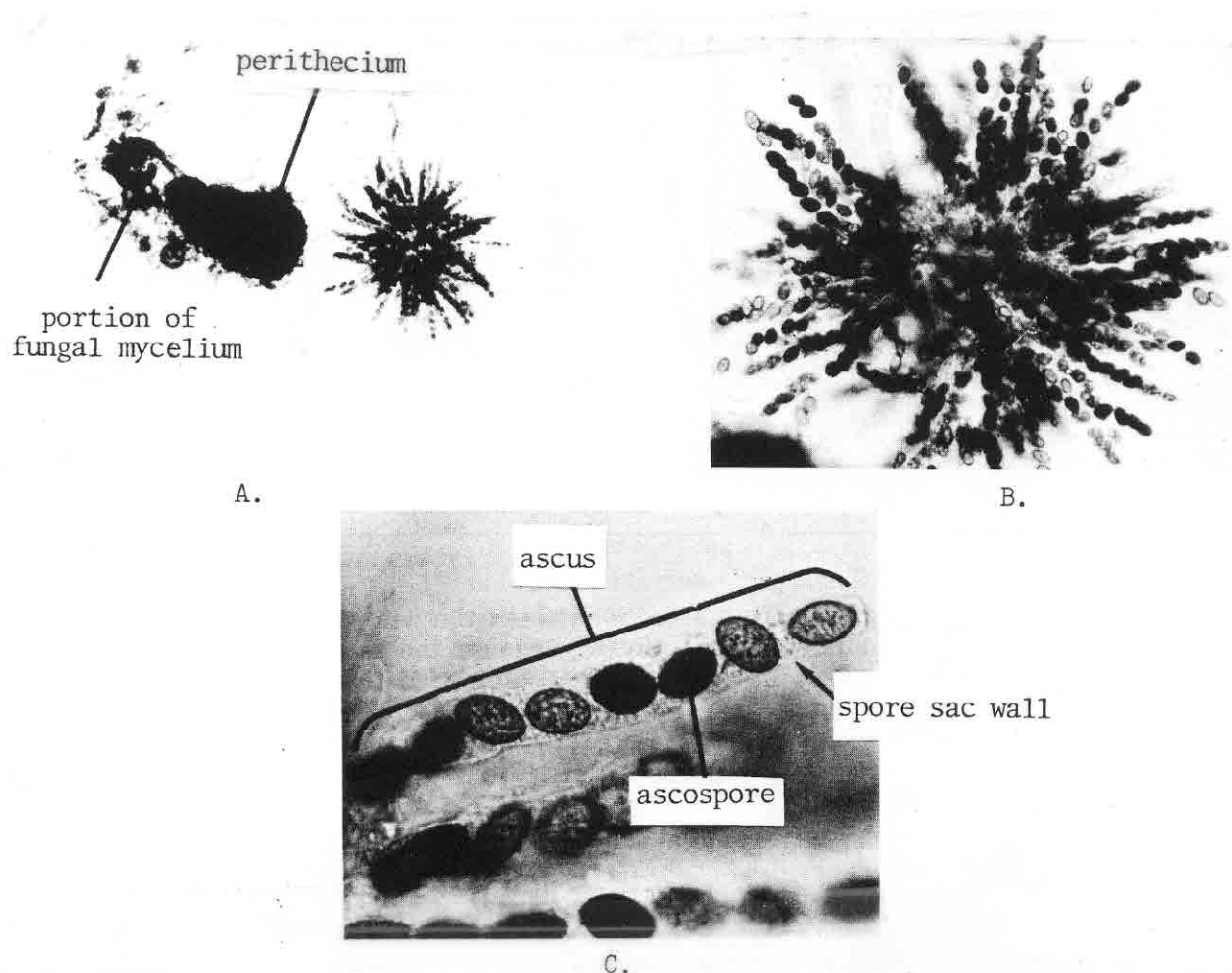
Figure 2.9. Photomicrographs of *Sordaria fimicola*. *A.* Squashed perithecium and spread asci. *B.* Close-up of spread asci. *C.* Close-up of tetratype ascus.

Data Collection

Properly squashed perithecia will eject their asci in a radial arrangement, like the spokes of a wheel. In collecting data it is best to start at one point, systematically moving in a clockwise or counterclockwise fashion, categorizing each ascus that can be clearly seen until a full circle has been completed. You will need to focus carefully to see all the spores in an ascus. Note that the ascus narrows on the end that connects it to the fungal mycelium. Locating the narrowed, broken end will allow you to properly classify any isolated asci that may be found on your slides.

❑ Your Lab Instructor will project several slides showing tetratype asci so you can start to learn the four different spore phenotypes. To be most successful at identifying spore phenotypes as you use the microscope to examine perithecial squashes be sure to&

    1. open the iris diaphragm so that you can distinguish the colors.
    2. keep light intensity low so you can see color differences better.
    3. focus up and down carefully to see all eight ascospores.
    4. avoid immature asci (their spores will have a <u>granular</u> appearance and very little color).
    5. seek your instructor's help until you become confident in your ability to identify the phenotypes.

❑ As you classify asci, write down in abbreviated form the sequence of spores within each ascus. For example, the ascus in 2.6 **E** could be represented as CCBBGGTT (from left to right) or, since all spore phenotypes are in duplicate due to the mitotic division after meiosis, more simply CBGT.

❑ Each individual should classify at least <u>30 asci</u> to insure an adequate sample size for the class data. Enter individual data in Table 2.1.

<u>Possible</u> <u>Types</u> <u>of</u> <u>Asci</u>

In addition to PD and NPD asci, you will see a variety of T asci, some with all four spore phenotypes and some with only two phenotypes in a 2:2:2:2 or a 2:4:2 arrangement. However, there are some restrictions on the types of two-phenotype asci that are possible. Specifically, in a cross between the two mutants, you can not observe any of the arrangements that result from the two wild type ´ mutant crosses. Black and gray only or black and tan only are not possible (either in a 4:4 or non-4:4 arrangement) because these arrangements require more wild type alleles than are available in a mutant ´ mutant cross. Likewise, colorless and gray only or colorless and tan only are not possible (either in a 4:4 or non-4:4 arrangement) because these arrangements require more mutant alleles than are available. In other words, two-phenotype asci can only include the phenotypes tan and gray or colorless and black.

❑ Go back through your asci descriptions and identify each ascus as either PD, NPD, or T in Table 2.1.

<div align="center">

PD asci are only

NPD asci are only

</div>

T asci are <u>all</u> others having two or all four phenotypes in a 2:2:2:2 pattern or a 2:4:2 arrangement of two phenotypes (but the two-phenotype asci must be gray-&-tan or colorless-&-black). Do not classify any asci having three phenotypes or disordered spores where phenotypes do not occur in pairs.

Table 2.1. Data collected from a genetic cross between the gray and tan spore strains of *Sordaria fimicola*. (Note: C = colorless, B = black, T = tan, G = gray spore).

Ascus Number	Phenotypes (i.e. TGBC)	PD, NPD or T?	Genotypes	Gray Spore Gene – Spores Recomb.	Gray Spore Gene – Spores Non-Recomb.	Tan Spore Gene – Spores Recomb.	Tan Spore Gene – Spores Non-Recomb.
1			g t - g t - g t - g t				
2			g t - g t - g t - g t				
3			g t - g t - g t - g t				
4			g t - g t - g t - g t				
5			g t - g t - g t - g t				
6			g t - g t - g t - g t				
7			g t - g t - g t - g t				
8			g t - g t - g t - g t				
9			g t - g t - g t - g t				
10			g t - g t - g t - g t				
11			g t - g t - g t - g t				
12			g t - g t - g t - g t				
13			g t - g t - g t - g t				
14			g t - g t - g t - g t				
15			g t - g t - g t - g t				
16			g t - g t - g t - g t				
17			g t - g t - g t - g t				
18			g t - g t - g t - g t				
19			g t - g t - g t - g t				
20			g t - g t - g t - g t				
21			g t - g t - g t - g t				
22			g t - g t - g t - g t				
23			g t - g t - g t - g t				
24			g t - g t - g t - g t				
25			g t - g t - g t - g t				
26			g t - g t - g t - g t				
27			g t - g t - g t - g t				
28			g t - g t - g t - g t				
29			g t - g t - g t - g t				
30			g t - g t - g t - g t				

PD Asci = _____   Spore Totals = _____   _____   _____   _____

NPD Asci = _____   Crossover Asci =   _____   _____

T Asci = _____   Non-crossover Asci =   _____   _____

Total Asci = _____   _____   _____

DATA ANALYSIS AND TRANSFORMATION

Your lab section will collaborate in analysis and transformation of data collected in this study. Be sure to obtain a complete copy of all of the data; you will need these data when you complete the worksheet associated with this chapter. The worksheet is concerned with providing answers to the following three key questions:

1.   Are the gray and tan spore genes linked or unlinked?

2.   What are the gene-centromere map locations of the gray and tan spore genes?

3.   How does the occurrence of multiple crossing over affect your attempts to map these two genes using tetrad analysis?

The following sections discuss each of these questions in more detail. Refer to this information as you complete the worksheet. Note: you will need to use the chi square test as part of the data analysis of this study. Read the section of the Statistical Reference Appendix devoted to this test (p. A52-A55).

Linkage or Non-Linkage

The criterion to be used in deciding if the two genes are linked or not is whether they assort independently; unlinked genes do, linked genes do not. As discussed earlier, if the tan and gray spore genes are unlinked, we expect equal numbers of parental ditype asci (PD) and nonparental ditype (NPD) asci. Determine the total number of PD and NPD asci observed by the whole class and use the chi square test to compare these numbers with the expected values assuming the genes are unlinked (see worksheet question 1).

❑   Complete Table 2.2 using the class data assembled from individual Tables 2.1.

Tetrad Analysis and Gene Mapping

Tetratype (T) asci result from crossing over between homologous chromosomes. However, as shown in Figures 2.6 and 2.8, some crossovers affect just one gene, while others affect both genes. In using tetrad analysis data to map the gray and tan spore genes, you must determine whether the alleles for one or both genes were switched by the crossover that produced each tetratype ascus you observed. Using the information that follows, each student should analyze the tetratype asci s/he recorded in Table 2.1 and record the correct number of recombinant and non-recombinant spores for each gene in that table. You will then combine individual data to estimate map locations for the two genes based on all of the class data.

We must assume that NPD and PD asci contain only non-recombinant ascospores (even though some of these asci resulted from multiple crossing over, as we shall discuss shortly). For each NPD and PD ascus you recorded in Table 2.1, place a 0 in the Recomb. spores and an 8 in the Non-recombinant spores column of both the tan and gray spore gene categories. In the case of the T asci, to determine which genes were affected by crossing over you must determine which genes show second division segregation.

Examine the genotypes for the spores in the ascus shown in Figure 2.6 **C**. If there is no crossover between a gene's locus and the centromere then according to Mendel's principle of segregation the gene's alleles will show first-division segregation. First division segregation produces a 2:2 arrangement of genotypes within the ascus for that gene (considering the original chromatids before the mitotic replication). A crossover between the gene's locus and the centromere will lead to second division segregation and the arrangement of genotypes within the ascus will be 1:1:1:1 or 1:2:1 for that gene. In

Figure 2.6 **C**, notice that the arrangement for the gray spore gene is **g⁺:g⁺:g :g**, but **t : t⁺: t⁺: t** for the tan spore gene. This observation indicates that during the meiotic division that produced this ascus a crossover occurred for the tan spore gene but not the gray spore gene. Had you observed this ascus, you would know that only the chromosome with the tan spore gene was changed due to the crossover and that the four recombinant ascospores resulting from that crossover should be associated with the tan spore gene. Therefore, in Table 2.1 you would place a 4 into the <u>Recomb.</u> spores and <u>Non-Recomb.</u> spores columns for the tan spore category and a 0 in the <u>Recomb.</u> spores and an 8 into the <u>Non-Recomb.</u> spores column for the gray spore category.

Look at the pattern of genotypes shown for the ascus shown in Figure 2.6 **D**. Which gene is showing second division segregation? Had you observed this ascus, you would know that only the chromosome with the gray spore gene was changed due to the crossover and that the four recombinant ascospores resulting from that crossover should be associated with the gray spore gene. Therefore, in Table 2.1 you would place a 4 into the <u>Recomb.</u> spores and <u>Non-Recomb.</u> spores columns for the gray spore category and a 0 in the <u>Recomb.</u> spores and an 8 into the <u>Non-Recomb.</u> spores column for the tan spore category.

Look at the pattern of genotypes shown for the asci shown in Figure 2.6 **E** and **F**. Which genes show second division segregation? Notice that all eight ascospores in these asci are recombinant: four are recombinant for the one gene, and four for the other gene. When you observe asci of these types, you would place a 4 in the Recomb. <u>and</u> Non-Recomb. spore columns of <u>both</u> gene categories.

This same approach is used whether one assumes that the genes are unlinked or linked. For example, in the ascus in Figure 2.8 **B**, the pattern of genotypes for the two genes indicate that both genes were affected by the crossover. This would occur if the gray and tan genes are located on the same side of the chromosome relative to the centromere and a crossover had occurred between the centromere and the gene locus nearest the centromere. If you had observed this ascus, you would place a 4 in the <u>Recomb.</u> spores and a 4 in the <u>Non-Recomb.</u> spores column for <u>both</u> gene categories, since both genes were affected by the crossover.

If you had observed the pattern of genotypes for the ascus shown in Figure 2.8 **C**, you would place a 4 in the <u>Recomb.</u> spores and the <u>Non-Recomb.</u> spores column for the gray spore gene and a 0 in the <u>Recomb.</u> spores and an 8 in the <u>Non-Recomb.</u> spores column for the tan spore gene, because only alleles of the gray gene are showing second division segregation. Verify that this is true.

❑ For each ascus you recorded in Table 2.1, enter the four spore genotypes in the space provided and determine the numbers of recombinant and non-recombinant spores for each gene.

❑ Complete Table 2.3 using the class data assembled from individual Tables 2.1.

❑ To estimate the map locations of the two genes, whether unlinked or linked, simply determine the total number of recombinant spores for each gene and divide by total spores observed (see worksheet question 2). **Note**: a check on your computations would be to ascertain that total spores observed for both genes (non-recombinant spores + recombinant spores) is the same number.

Comparison with Published Map Locations

Published gene-centromere map distances for the two genes are approximately 27 map units (Olive et al., 1975) for the tan and 33 map units for the gray spore gene (Olive 1956). You should use the chi square test to determine if your estimated map locations differ significantly from these values. Since map units represent the percentage of all the spores that are recombinant, you might be inclined to use *non-recombinant and recombinant* spores as the categories in the chi square test. However, since the observed values in your chi square categories represent your sample size, and samples must be

independent, you must select categories that reflect the amount of real (independent) information you have collected. Since the number of spores, (recombinant or not) is really a function of the number of asci (crossover or not) you observed for that gene, and since spore numbers are not independent (why?), you must use *crossover and non-crossover* asci as test categories. So, for example, if a gene is 37 map units from its centromere, recognizing that each crossover produces one-half recombinant and one-half non-recombinant spores, you would expect 74% crossover asci and 26% non-crossover asci for that gene. Your observed values will be the actual number of crossover asci and non-crossover asci for that gene. The expected values would be the total number of asci observed, times 0.74 for the expected crossover asci and times 0.26 for the expected non-crossover asci.

❑   Complete Table 2.4 using the class data assembled from individual Tables 2.1.

Table 2.2. The number of Parental Ditype (PD), Non-Parental Ditype (NPD), and Tetratype (T) asci from a genetic cross between the tan and gray spore strains of *Sordaria fimicola* .

Ascus Type	Number Counted
Parental Ditype (PD)	
Non-Parental Ditype (NPD)	
Tetratype (T)	

Table 2.3. Recombinant and non-recombinant spores for the tan and gray spore genes of *Sordaria fimicola* from a genetic cross between the two mutant strains.

	Tan Spore Gene	Gray Spore Gene
Recombinant Spores		
Non-recombinant Spores		
Total Spores		

Table 2.4. Observed and expected crossover and non-crossover asci for the tan and gray spore genes of *Sordaria fimicola* from a genetic cross between the two mutant strains.

	Tan Spore Gene	Gray Spore Gene
Observed Crossover Asci[1]		
Observed Non-crossover Asci[2]		
Expected Crossover Asci[3]		
Expected Non-crossover Asci[4]		

[1] T asci with crossover for gene.

[2] PD and NPD asci and T asci without crossover for gene.

[3] Total asci examined ∗ 0.54 for the tan spore gene of total asci examined ∗ 0.66 for the gray spore gene.

[4] Total asci examined ∗ 0.46 for the tan spore gene of total asci examined ∗ 0.34 for the gray spore gene.

Testing a Complete Genetic Model- Numerical Simulation of Meiosis

You will now use the published gene-centromere map distances to predict the frequencies of PD, NPD, and T asci assuming that the tan and gray spore genes are phisycally unlinked. Consult Figure 2.10 with respect to the following discussion.

If the distance between the tan locus and its centromere is 27 map units, then the frequency of meiotic divisions with a single crossover between the tan locus and its centromere is equal to 0.54, and the frequency of meiotic divisions with no crossover for that gene will be 0.46 (1.00 - 0.54). If the distance between the gray locus and its centromere is 33 map units, then the frequency of meiotic divisions with a single crossover between the gray locus and its centromere is equal to 0.66, and the no-crossover frequency for the gray gene would then be 0.34 (1.00 - 0.66). The predicted frequency of asci resulting from <u>no</u> crossovers in meiosis (i.e., <u>PD and NPD asci</u>) would than be:

frequency of meiotic divisions with no crossover between the <u>tan</u> gene and its centromere		frequency of meiotic divisions with no crossover between the <u>gray</u> gene and its centromere		
(0.46)	´	(0.34)	=	0.16

Since unlinked genes show independent assortment, half of these asci resulting from no cross-over would be PD, and the other half NPD. Therefore, predicted frequency of PD asci = predicted frequency of NPD asci = .16/2 = .08

TOTAL MEIOTIC DIVISIONS

	Expected Frequency of Crossovers	Expected Frequency of Non-crossovers
27 map units	0.54	0.46
33 map units	0.66	0.34
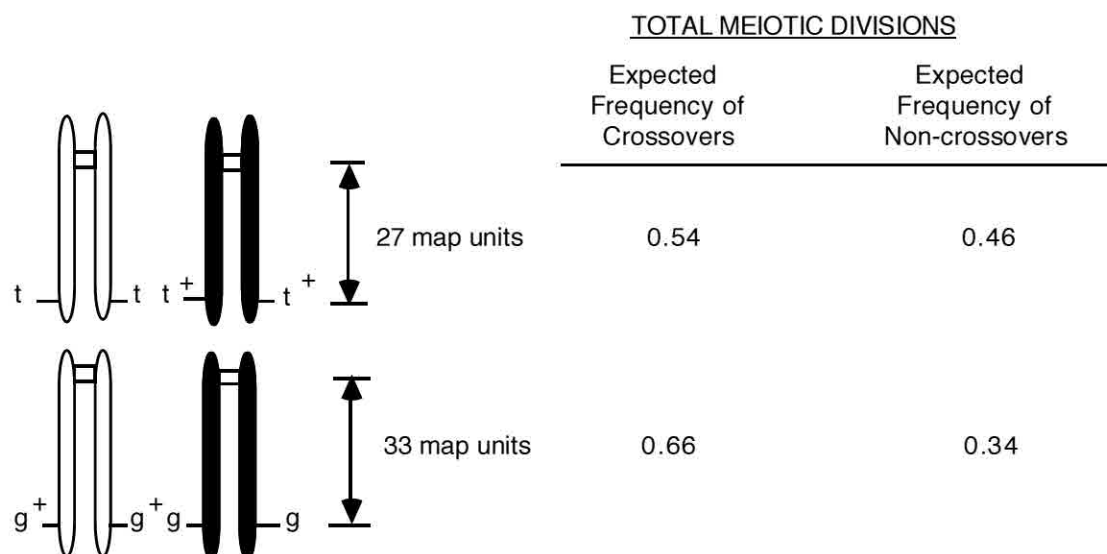
Figure 2.10. The relationship between map unit distance and the expected frequencies of meiotic divisions with and without single crossovers for two unlinked genes

A single crossover between <u>either</u> gene and its centromere or a simultaneous single crossover between both genes and their centromeres would all produce T asci. Therefore, the predicted frequency of T asci would be the sum of the products of the independent probabilities of all three of these events, or

predicted frequency of T asci =	(.54 ´ .34) +	(.66 ´.46) +	(.54 ´ .66)	= .84
	tan crossover only	gray crossover only	tan & gray crossovers	

❑ You have now modeled (simulated in a simplified way) the process of meiosis in *Sordaria*. Is the model a good description of reality, that is, do its predictions agree with real observations? Test this genetic model by performing the chi square test.

Multiple Crossover as an Error Source in Tetrad Analysis

After you have completed this analysis you should consider again the occurrence of multiple crossing over and its influence on your mapping attempts. We have assumed that all PD and NPD asci result from meiotic divisions without crossover and that all spores in PD and NPD asci are non-recombinant. Yet, as shown in Figure 2.6 and 2.8, some multiple crossovers produce PD and NPD asci. Did your chi square analysis of the observed frequencies of the three ascus types (PD, NPD, and T) show significantly more PD and NPD and proportionally fewer T asci than expected based on single crossovers?

Because of the production of PD and NPD asci by multiple crossing over some of the spores in your Non-Recomb. Spores columns of Table 2.1 are really recombinant spores. Also, some T asci contain eight recombinant spores due to multiple crossovers, but only show second division segregation for a single gene (see Figure 2.8D). Since map units are percent recombinant spores, multiple crossovers cause us to underestimate map units.

Because each crossover is an independent event, the probability of two crossovers occurring together is the product of their independent probabilities. Thus, the probability of multiple crossing over is small if genes are close together, but increases rapidly as genes are further apart. Geneticists minimize the problem of multiple crossing over by only mapping genes that are close together. The gene-centromere map units for the gray and tan genes cited above were obtained by adding together map units for other linked genes located between these genes and their centromeres. Thus, you may find that your estimates may differ significantly from these values.

REFERENCES AND SUGGESTED READINGS

Campbell NA, Reece JB. 2008. Biology. 8th ed. San Francisco, CA: Benjamin/Cummings Publishing Co.

Cassell P, Mertens TR. 1968. A laboratory exercise on the genetics of ascospore color in *Sordaria fimicola*. The American Biology Teacher 30(5): 367-372.

Fincham JRS. 1971. Using fungi to study genetic recombination. Oxford Biology Reader no. 2. London: Oxford University Press. (Discusses the current hypotheses to explain the occurrence of the aberrant asci that you may have observed in this laboratory.)

Fincham JRS, Day PR, Radford A. 1979. Fungal genetics. Oxford: Blackwell Scientific.

Fincham JRS. 1983. Genetics. Boston: Jones and Bartlett. (Includes several chapters about fungal genetics and tetrad analysis.)

Olive LS. 1956. Genetics of *Sordaria fimicola*. I. ascospore color mutants. American Journal of Botany 43:97-107.

Olive LS, El-Ani AS. 1975. Genetics of *Sordaria fimicola*. IX. Linkage group II. American Journal of Botany 62:166-171.

Rushforth SR. 1976. The plant kingdom. New Jersey: Prentice-Hall, Inc.

Suzuki DT. 1989. An introduction to genetic analysis. New York: W.H. Freeman. (Chapter 6 includes information about gene linkage and tetrad analysis in fungi.)

Sordaria GENETICS WORKSHEET

Name: _____   Lab Instructor: _____

1.   Are the tan spore and gray spore genes linked or not? Use the data included in Table 2.2 to do the chi-square test to address this question by comparing the numbers of PD and NPD asci.

CLASS TOTALS

Ascus Type	Observed (O)	Expected (E)*	(O – E)	$(O – E)^2$	$(O – E)^2 / E$
PD Asci					
NPD Asci					
TOTALS =				$c^2 =$	

* See table 2.2.

H_o:

H_a:

Test Statistic (C^2) =   **Tabular Statistic ($a =$, d.f. =   ) =**

Conclusion:

2.   Using the data included in Table 2.3, estimate the gene centromere-map unit distances for the tan and gray spore genes.

<u>Tan Spore Gene:</u>

<u>Gray Spore Gene:</u>

3.   Answer the following question assuming that the tan and gray spore genes are <u>linked</u> as shown below:

Map Units
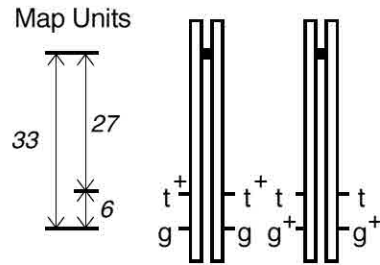
A.   What is the probability of a single crossover between the tan gene locus and the centromere?

B.   What is the probability of a double crossover between the tan and gray gene loci?

C.   Carefully diagram in the space below a crossover that would produce the following ascus starting with the chromosome arrangement shown above.
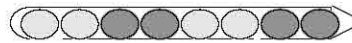
   or   **2 tan: 2 gray: 2 tan: 2 gray**

4.   A.   In the mutant–wild type cross, you used the concept of second division independent assortment of alleles in determining whether a crossover had occurred, and thus, whether spores were recombinant or non-recombinant (review page 32). One can also determine whether alleles are showing second division independent assortment in the mutant–mutant cross. Look at the genotypes you filled in for the T asci in Figures 2.6 and 2.8. Consider each gene separately. Note that in 2.6 C, the gray alleles are in a 2:2 arrangement; the tan alleles are 1:2:1. For which gene has a crossover occurred (with the alleles showing second division independent assortment)?

                                                                            _____

B.   Note in the T asci shown in Figure 2.6, as well as those in Figure 2.8, that the arrangement of each gene's alleles reflects whether a crossover has occurred between that gene and its centromere. Starting with the chromosome arrangement illustrated in question 3 (a mutant–mutant cross that assumes the tan and gray spore genes are linked), determine whether the tan, the gray, or both genes show second division independent assortment (i.e. experienced a crossover event) in the following Tetratype asci. Start by writing the spore genotypes for each ascus. Remember to consider each gene separately.

Spore arrangement in ascus	Is there evidence for any of the genes (gray, tan, or both) having been involved in a cross over event? Which ones?
2 black: 2 gray: 2 colorless: 2 tan	
2 gray: 4 tan: 2 gray	
2 black: 2 tan: 2 gray: 2 colorless	
2 gray: 2 tan: 2 black: 2 colorless	

C.   Would your answers to B be different if the genes were not linked in this mutant–mutant cross? Why or why not?

NOTES

CHAPTER 3 – GENETIC CONTROL OF BIOCHEMICAL PATHWAYS

SYNOPSIS

In this laboratory you investigate the biosynthetic pathway for the production of the red pigment, prodigiosin, in the bacterium *Serratia marcescens.* By performing "feeding" trials, you examine the genetic control of this pathway. This laboratory also gives you experience with sterile methods for manipulating microorganisms. First, you will set up the pairwise trials and then several days later you will complete the lab, by collecting the results of the trials.

LEARNING OBJECTIVES

Conceptual

1. Describe a typical biochemical pathway in terms of precursor, intermediate, and final product molecules and understand how pathways are controlled by specific genes.

2. Explain how mutant (auxotroph) strains can be produced from a wild type (prototroph) strain, and how they differ from the wild type strain.

3. Describe the four parts of the explanatory system for the interpretation of syntrophic feeding trials.

4. Use the explanatory system to interpret a set of data from syntrophic feeding trials and construct a table showing the most reasonable pathway.

5. For each auxotroph strain in a given pathway, show which pathway substances are secreted, which substances are missing, and the feeding relationships to other strains.

Methodological

1. Use sterile methods to transfer and plate bacteria.

2. Draw biochemical pathways using the described conventions.

READING ASSIGNMENT

Read this chapter in the lab manual.

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

1. Why is each mutant strain in this study unable to produce the pigment prodigiosin?

2. What does it mean if we say that one mutant strain is able to "feed" another mutant strain?

3. What does it mean if we say that one mutant strain is "fed by" another mutant strain?

4. What kind of substance is the "feeding" strain providing to the "fed" strain?

5. How will you know if a mutant strain has been "fed" in a cross-feeding trial?

6. Can the wild type strain "feed" other strains? Explain your answer.

Jon C. Glase

Revised June 2010
Mark A. Sarvary

INTRODUCTION

The terms **genotype** and **phenotype** are much used by geneticists (Figure 3.1). An individual's genotype is the specific alleles present in the individual's chromosomes for whichever genes are being considered. An individual's phenotype is the outward expression of those alleles in terms of either the structural appearance of the organism or its functional capabilities. The individual's genotype determines its phenotype via the gene's control of chemical reactions within cells.
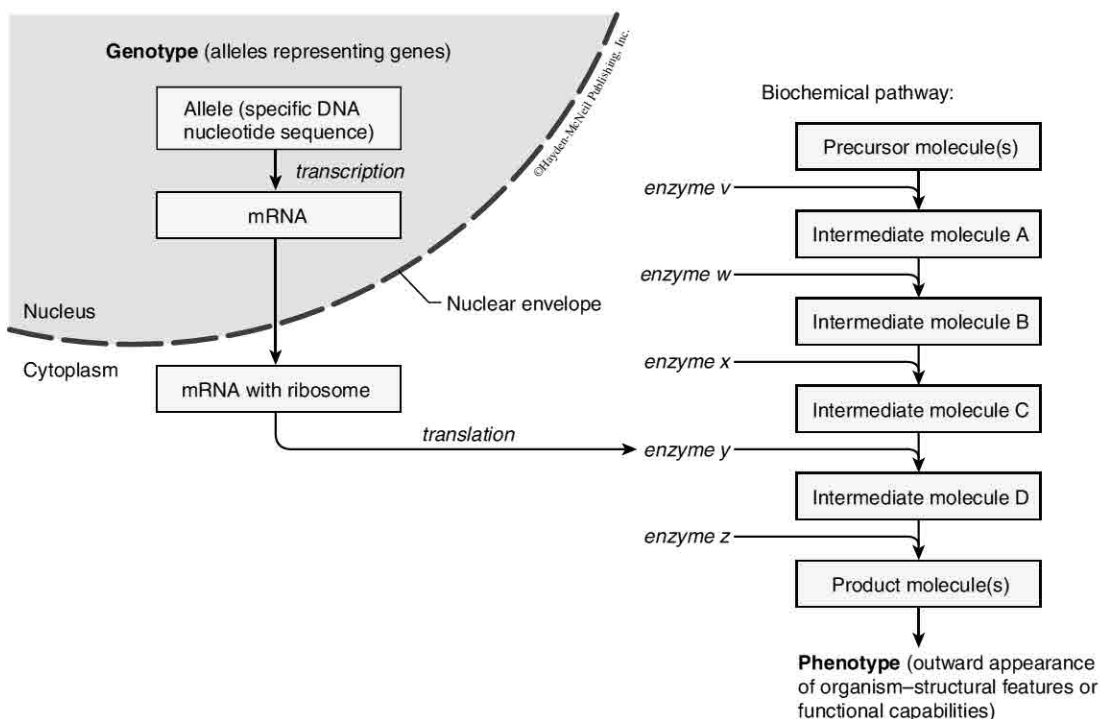
Figure 3.1. How the genotype of a eukaryotic cell determines its phenotype via the genetic control of the cell's biochemical pathways. Same scheme can apply to prokaryotes if the nuclear envelope is absent.

Biologists can now relate many of an organisms structural/functional features (its phenotype) to specific chemical reactions that have occurred within its cells. Within cells, chemical reactions are typically coupled together in what are called **biochemical pathways**. A typical biochemical pathway begins with one or more **precursor** molecules, the raw materials for the pathway which are either obtained by the cell from the environment or may be the product of some other pathway. These precursors are converted through several different reactions into **intermediate molecules** (A-D) until eventually a **final product** molecule(s) results. Product molecules are then expressed in the cell's phenotype, either as a structural cell feature or functional capability.

It is through control of the biochemical pathways within its cells that an individual's genotype determines its phenotype. Each of the reactions within the pathway is catalyzed by a specific enzyme. The information needed for each enzyme's production resides in the DNA nucleotide sequence for the gene coding for the enzyme. The DNA nucleotide sequence or **allele** for the gene produces a complimentary RNA nucleotide sequence (the messenger RNA or mRNA) which couples with ribosomes in the cytoplasm and directs the synthesis of the enzyme. The mRNA nucleotide sequence determines the amino acid sequence of the enzyme. The presence of the appropriate enzyme allows a specific step of the pathway to be completed. All of the genes coding for all of the enzymes involved in the pathway must be functioning normally (must be producing normal, functional enzymes) if the pathway is to be complete and product formed.

HOW BIOCHEMICAL PATHWAYS ARE STUDIED

The basic method for studying the genetic control of biochemical pathways is elegantly simple. One begins with a wild type or **prototroph** strain of the organism, whose cells <u>can</u> produce the end product of the biochemical pathway being studied. The prototroph strain has cells with a functional pathway, because it has wild type alleles for each of the genes controlling the pathway. The prototroph strain is then treated with a mutagen such as x-radiation or UV light. The mutagen produces mutant or **auxotroph** strains. Auxotroph strains have blocks in the pathway due to the conversion of a wild type allele to a mutant allele for any of the genes controlling the pathway. For example, Figure 3.2 shows a simple linear biochemical pathway with 4 steps; precursor **A** is changed through intermediates **B**, **C**, and **D** to become the end product. Each step in the pathway is catalyzed by a different enzyme (*w, x, y, z*) and each enzyme is coded for by a different gene (*W, X, Y, Z)*. Each of the four auxotroph strains (1 - 4) has a block at a different step in the pathway because of a mutant allele for one of the four genes controlling the pathway. In auxotroph strain 1 the mutagen changed the nucleotide sequence in gene 4, converting the wild type allele to a mutant allele. The mutant allele codes for altered enzyme, z^*, instead of the normal enzyme *z*. The altered enzymes, z^*, can not catalyze the conversion of **D** to end product and, thus, auxotroph 1 is blocked in that step of the pathway. As shown, the other three auxotroph strains are blocked at other steps due to mutant alleles for the other three genes controlling the pathway. Notice that even though each auxotroph is blocked at a different step, the end result is the same -- no end product is produced.

The terms prototroph and auxotroph derive from the pioneering work done on the genetic control of biochemical pathways in *Neurospora* by George W. Beadle and Edward L. Tatum (Campbell and Reece, 2008, p. 326-327). The prototroph strain of *Neurospora* was able to grow successfully on a defined medium called minimal media. Prototroph means *self feeder.* The prototroph strain was exposed to X rays to produce numerous auxotroph strains that could grow on minimal media only if it was supplemented with a specific nutrient unique for each different auxotroph. Auxotroph means *increased feeder*, referring to the fact that its nutrient requirements had been increased by mutation.

The major questions we want to answer in studying a biochemical pathway are the following:

1.   How many reaction steps are in the pathway?

2.   What is the shape of the pathway?

3.   Which genes control which steps in the pathway?

The basic approach is to sequence the auxotroph strains in the same order as the reaction steps in which they are blocked. We can do this because auxotrophs differ in one very important way from the prototroph strain. When the wild type strain has produced all the end product that is required by the cell, the presence of adequate end product turns off the pathway genes. The genes stop producing their enzymes and the reaction steps within the pathway are halted. When more end product is required the genes are turned on and the pathway again becomes functional (how genes are turned off and on is discussed in your textbook). Because auxotroph strains can not produce end product their pathway operates continuously. As a result each auxotroph produces large quantities of the intermediate substance that is substrate for the reaction step which is blocked in the auxotroph strain. So much of this blocked intermediate is produced that the auxotroph strains typically secrete it from their cells. Thus, Strain 1 in Figure 3.2 would secrete the intermediate substance **D**.

❑   In Figure 3.2, circle the intermediates that the other strains would secrete.

The auxotroph strains can be sequenced in the order of their blocks in the pathway by determining which strains can use the other strain's secreted intermediate substances to complete their pathway. **Syntrophic feeding** occurs when one strain uses the secreted intermediate substance of a different strain to complete its blocked pathway. If a strain can use the secreted intermediate of another strain, it is said to be fed by that strain. If an auxotroph strain is fed by another auxotroph strain, then we know that the "fed" strain must have its block at a step in the pathway <u>before</u> the blocked step in the feeder strain. For example, in Figure 3.2, auxotroph 1 secretes substance **D**. **D** will allow all other strains to complete their pathways, because any **D** that strains, 2, 3, or 4 can get will enter their pathways somewhere after the step in which they are blocked. However, strain 4 can not feed strain I (or any other strain) because the block in strain I occurs after the block in strain 4. Thus any **A** that enters strain 1's pathway from strain 4 will simply be blocked at step 4 and strain 1 still will not be able to produce end product.

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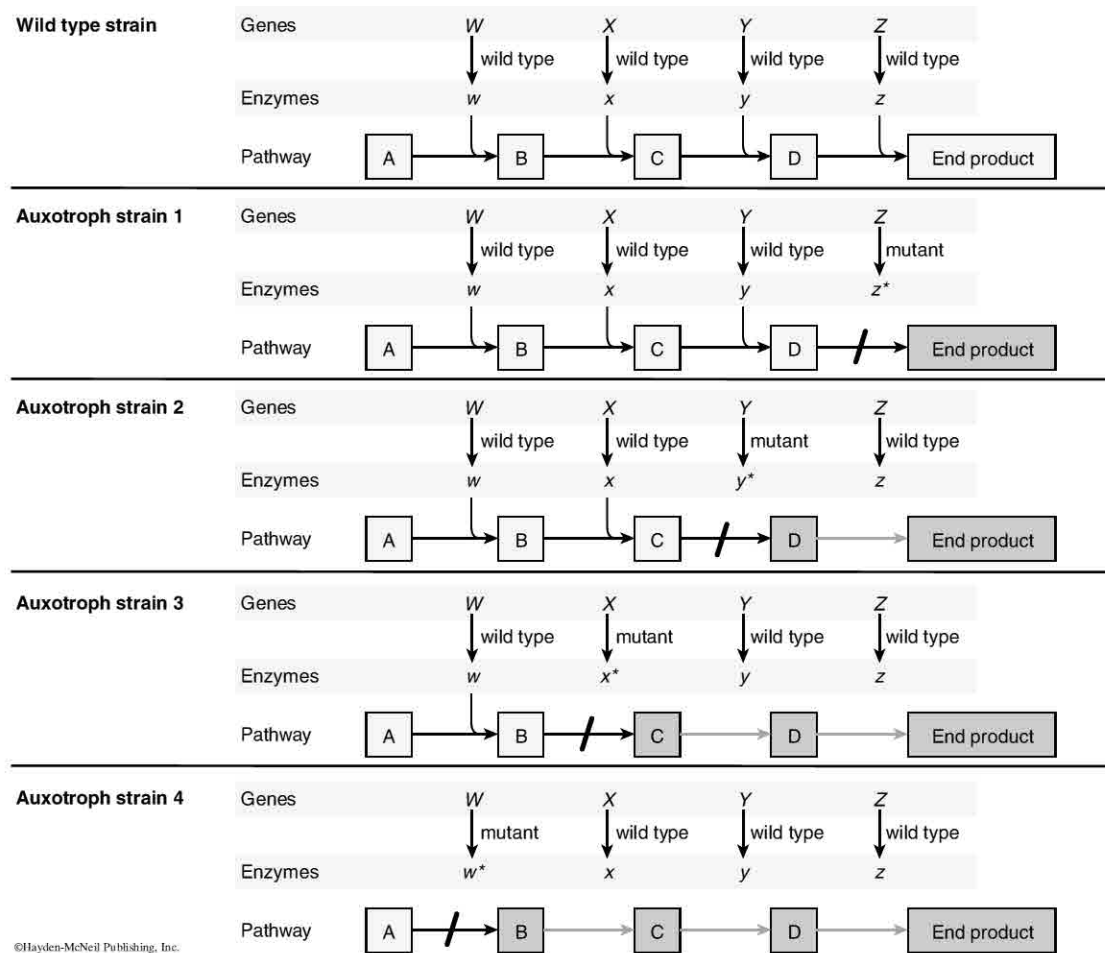
Figure 3.2. Four auxotroph strains (1 - 4) resulting from hypothetical blocks in a simple linear pathway due to a mutation in one of the four genes controlling the pathway. Note: *w.t.* = wild type allele, *mut.* = mutant allele. Substances in shaded boxes are not produced by the auxotroph strain.

EXPLANATORY SYSTEM FOR AUXOTROPH FEEDING TRIALS

Through numerous studies of biochemical pathways using syntrophic feeding between auxotroph strains, biologists have verified several hypotheses that can now serve as an explanatory system to help interpret observations of new pathways. Three elements of the explanatory system can be stated as follows:

1. If an auxotroph strain has its block in the first reaction step of the pathway, then it will be fed by all other auxotroph strains, but can feed none.

2. If an auxotroph strain has its block in the last reaction step of the pathway, then it will feed all other auxotroph strains, but be fed by none.

3. For strains with blocks in steps between the first and last step, the strain with its block close to the beginning of the pathway will be fed by all strains with blocks at later steps; conversely, strains with blocks closer to the end of the pathway will not be fed by strains with blocks earlier in the pathway.

To be certain that you understand why these facts are true, apply them to the strains in Figure 3.2.

Biochemical pathways can be branched. Consider the pathway shown in Figure 3.3. This pathway has six reaction steps each catalyzed by a different enzyme. A separate gene codes for each enzyme. Each of the six auxotroph strains are blocked at a different step in the pathway because they each possess a mutant allele for one of the genes and, thus, can not make the necessary enzyme for that step. The pathway includes a closed loop formed when precursor A is split to form B and C which are converted, respectively, into D and E. Intermediates D and E are then recombined to form F; F becomes G; and finally G is converted into the pathway's end product. The final element of the explanatory system deals with branched pathways.
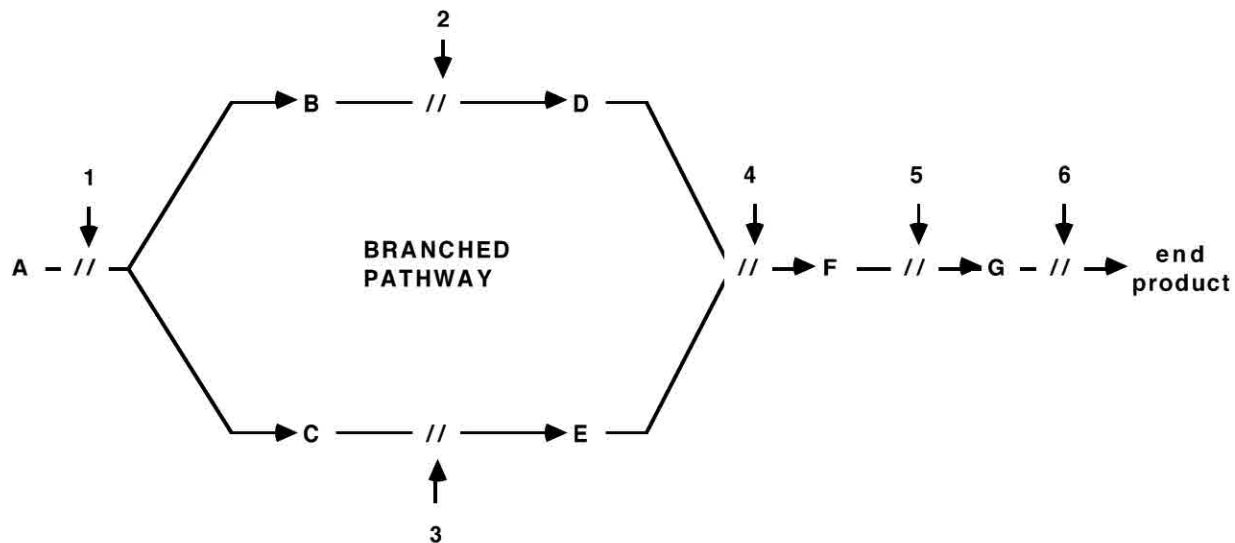
Figure 3.3. A branched biochemical pathway with six reaction steps. Each of the auxotroph strains (1 - 6) has a block in the step shown but is functional for all other steps. A-G are the pathway substances.

4. If a pathway is branched, then mutual feeding will occur between all strains with blocks on opposite branches of the pathway. In mutual feeding, one auxotroph feeds another strain and is, in turn, fed by that same strain.

Consider strains 2 and 3 in Figure 3.3. Strain 2 is blocked in the upper branch of the pathway and cannot convert B to D; consequently substance B accumulates and is secreted by strain 2's cells. However, because the other branch of the pathway is functional, C is converted into E. Since there is no D to combine with E, E also accumulates and is secreted by 2. The reverse is true for strain 3. Strain 3 can convert B to D but is blocked in the C to E step. Therefore, strain 3 secretes both C and D. Mutual feeding results because 2 can use the D secreted by 3 to combine with its own E to make F and 3 can use the E secreted by 2 to combine with its own D to make F. Since both strains are functional for the other pathway steps, F is converted through the last two steps into the end product. Mutual feeding always occurs between auxotrophs with blocks on the opposite arms of a branched pathway.

Table 3.1. Important features of the six auxotroph strains in the pathway shown in Figure 3.3.

Auxotroph Strain	Substance(s) secreted due to block	Substance(s) missing due to block	Strain(s) that it feeds	Strain(s) that feed it
1	A	B,C,D,E,F,G	None	2, 3, 4, 5, 6
2	B,E	D,F,G	1, 3	3, 4, 5, 6
3	C,D	E,F,G	1, 2	2. 4, 5, 6
4	D,E	F,G	1, 2, 3	5, 6
5	F	G	1, 2, 3, 4	6
6	G	None	1, 2, 3, 4, 5	None

❑   Table 3.1 summarizes several important features of each auxotroph in the pathway shown in Figure 3.3. Be certain you can reconcile this table with the pathway and the explanatory system we have discussed so far. Do this before going on.

PRODIGIOSIN SYNTHESIS IN *Serratia marcescens*

Today you will use this explanatory system to study the genetic control of a biochemical pathway that produces an end product called **prodigiosin**. Prodigiosin is a deep red pigment found within the bacterium *Serratia marcescens*. Because of this pigment molecule, wild type *Serratia* is a deep red color. You will study five auxotroph strains of *Serratia*, each blocked at a different step within the pathway, and each a different color. The auxotrophs were produced by UV light treatment of the wild type. Strain designation and coloration are shown in Table 3.2.

Table 3.2. *Serratia marcescens* strains and their coloration.

<u>Strain</u>	<u>Coloration</u>
1	**Purple**
2	**Light Orange**
3	**Pink**
4	**Orange**
5	**Light Pink**
Hy (wild type)	**Dark Red**

You will be performing pair-wise syntrophic feeding trials in order to determine which secreted intermediates will allow completion of the prodigiosin pathway. In the study of many pathways, sophisticated biochemical methods are needed to determine if the pathway has been completed and the end product produced. You will visually determine if the pathway is complete by noting if the auxotroph strain has developed the deep red phenotype of the wild type strain. You will then use the explanatory system to determine the pathway's shape and the relative position of the blocked step in the pathway of each auxotroph strain.

PROCEDURE

Day One

Note: You need to use good sterile technique in this study because many other organisms (bacteria and fungi) will grow on your plates. Also, *Serratia* can cause skin irritations in humans, so use care in handling it and wash your hands with warm soapy water when you finish.

Note: The nutrient agar medium used to culture *Serratia* consists of 5 g peptone, 10 ml glycerol, 15 g agar, in 1 liter of water.

❑ Work in pairs for this study. Each group will need the following materials:

5 stock plates with the *Serratia* auxotroph strains shown in Table 3.2.

1 stock plate of the *Serratia* wild type H_y strain

16 sterile agar plates

2 inoculating loops

2 alcohol lamps or Bunsen burners

markings pens, masking tape, 95% ethanol for sterilizing work space

❑ Disinfect your work area by wiping down the surface with a paper towel soaked in 95% ethanol.

❑ Each of the five auxotrophs will be plated next to all others in pairwise combinations (see Table 3.3). Therefore, you will need 10 feeding-trial plates. Label these plates on the bottom to indicate which strains should be plated on each side. An example follows:
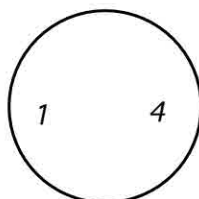
❑ You will also need a color reference plate of the wild type and each auxotroph. These 6 plates should be labelled on the bottom to indicate which strain is plated there. An example follows:
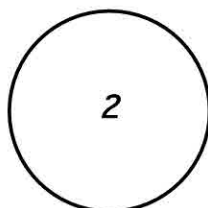
❑ To set-up the feeding-trial plates:

1. all group members should get experience in setting up some of these plates.

2.   place the plate to be streaked with the two auxotroph strains on the streak pattern template shown in Figure 3.4 A.

3.   have the two appropriate stock plates nearby.
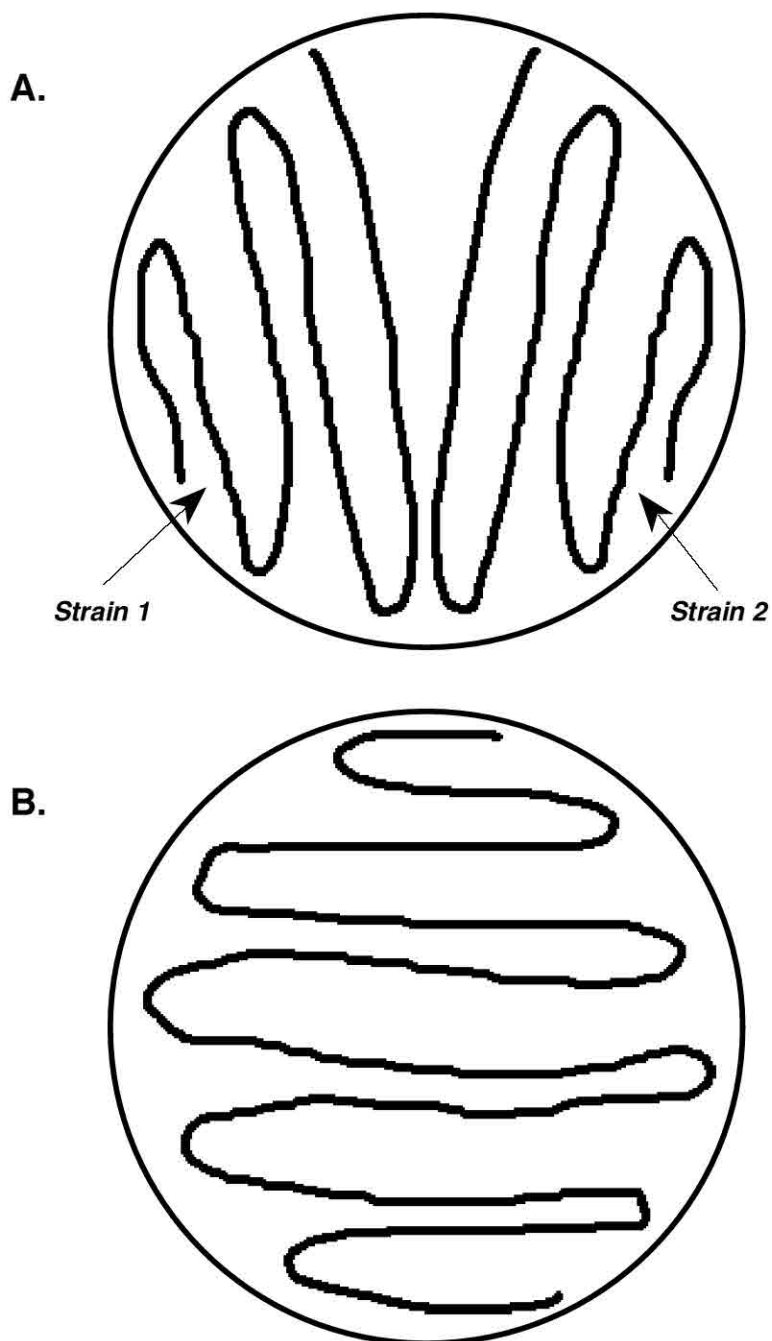
Figure 3.4. Streak patterns for (A.) the feeding-trial plates and (B.) the color reference plates.

4. flame the inoculating loop in the alcohol flame.

5. lift the lid of the stock plate slightly and cool the loop's tip by touching it to the inner lid's sterile surface for 5 seconds.

6. scrape the loop's tip across the agar surface where *Serratia* is growing.

7. quickly transfer the inoculum to the feeding trial plate resting on the streak pattern template.

8. lift the lid only enough to insert the loop and carefully move the loop according to the template to form one-half of the "V" pattern on the plate.

    Note: you will not be able to see the inoculum that you have plated. When plating bacteria, a little goes a long way.

9. reflame the loop and transfer the other auxotroph strain to form the other one-half of the "V" streak pattern as in steps 5-8 above.

    Note: do not overlap the two strains at the apex of the "V".

10. continue with this procedure until all 10 feeding trial plates have been setup.

❑   To setup the Color Reference plates:

1. use sterile technique, as described for the feeding trial plates, to initiate the 6 color reference plates.

2. use the streak pattern shown in Figure 3.4 B to obtain a uniform distribution of growth.

❑   On the lid of each plate record your names, date of inoculation, and your lab section # and room. Tape the lids to the plates using two small pieces of masking tape. Tape your plates in 4 stacks of 4 plates.

❑   Give the plates to your instructor. They will be returned in two days for data collection.

❑   Wipe down your work area with 95% ethanol and thoroughly wash your hands using a disinfectant soap.

Day Two (Thursday, July 15, 2010)

DATA COLLECTION

When you return to record your data, observe the feeding-trial plates carefully. Any mutant strain that has turned from its original color (compare with the color reference plate of that strain) to bright red (compare with the wild type reference plate) has successfully completed the biochemical pathway for the production of prodigiosin. Note: Some feeding will be very subtle and you will need to look carefully to be able to see the red color. Proceed as follows:

❑   For any plate where one or both of the mutants is not clearly being fed, remove the lid and examine the culture carefully with a binocular stereoscopic microscope at about 20x magnification. Note: *Serratia* is not a spore-forming bacterium, so it is safe to remove the lid as long as you do not touch the culture.

❑ Look especially at the border of the streak where the two mutants are closest (usually near the apex of the "V"). Do you detect any red wild type coloration? Compare with the borders of reference plates for both the mutant and wild type strains.

❑ All group members should look at each plate to arrive at a consensus on the group's results.

❑ Add your results to a data table for class results and keep your plates until all conflicts have been resolved. Resolved class data can be entered in Table 3.3.

❑ Dispose of the plates as directed by your laboratory instructor. Wipe down your work area with alcohol and thoroughly wash your hands with disinfectant soap.

Table 3.3. Class results of feeding trials between mutant strains of *Serratia marcescens*. A + sign in a square of the matrix indicates that prodigiosin was produced; a − sign indicates that it was not.

Strain Being Fed

	1	2	3	4	5
1	—				
2		—			
3			—		
4				—	
5					—

Strain Serving as Feeder

❑ Use the explanatory system to construct a pathway that best fits the data in Table 3.3. Diagram this pathway below, using the same format shown in Figure 3.3.

❑   Complete Table 3.4 for the proposed prodigiosin pathway.

Table 3.4. Important features of the five auxotroph strains in the prodigiosin pathway of *Serratia marcescens*.

Auxotroph strain	Substance (s) secreted due to block	Substance (s) missing due to block	Strain(s) it feeds	Strain(s) that feed it
1				
2				
3				
4				
5				

REFERENCES AND SUGGESTED READINGS

Campbell NA, Reece JB. 2008. Biology. 8th ed. San Francisco, CA: Benjamin/Cummings.

Lehninger AL. 1985. Principles of biochemistry. New York: Worth Publishers, Inc.

Morrison DA. 1966. Prodigiosin synthesis in mutants of *Serratia marcesens*. Journal of Bacteriology 91:1599-1604.

Mody RS, Heidarynejad V, Patel AM, Dave PJ. 1990. Isolation and characterization of *Serratia marcescens* mutants defective in prodigiosin biosynthesis. Current Microbiology 20(2):95–104.

Srb AM. 1971. Genes and metabolic pathways. In: Dethier VG and others (consultants). Topics in the study of life. New York: Harper and Row.

PRACTICE PATHWAYS—before you leave lab today, be sure you can construct the correct pathway given data such as those presented in Data Set 1 and 2 on the following pages.

Suggested Techniques[1]

Several tips suggested here may help you clarify some of the puzzling issues in solving the pathways.

1.   Each mutant has only one single mutation that affects a specific step in the pathway.

2.   When you are checking a mutual feeding relationship draw two identical pathways side by side to see if one mutant can feed the other and vice versa.

3.   The substances missing due to a block include all those intermediates downstream from the blocked point.

4.   The blocks of mutual feeders are located at the opposite arms of the branched pathway.

5.   If there is a block in one arm of the branched pathway, the substrate at the <u>end</u> of the other arm(s) will also accumulate in addition to the substrate immediately upstream from the block.

---

6. When you analyze data on a table that looks like Table 12.3 add the number of all the "+" of each row and record them individually at the right of the table. The higher the number of a mutant strain the more downstream of the pathway the block is located.

7. A "zero" in tally of "+" signs means the pathway has only one entry point. If there is no zero in the tally, the pathway is an "open" one, meaning that two or more substrates are required to start the pathway.

8. In addition, you may find that filling in a table such as the one shown below is helpful for you to more easily determine the shape and order of mutants in a biochemical pathway*. Data Set 1 on pg. 59 was used to fill in this table. Note how the beginning and end of the pathway is obvious. If the pathway begins open, then no strain would have <u>None</u> in the Feeds column.

    Strains listed in the left-hand column will precede a particular strain, and those in the right-hand column will follow that strain in the pathway. Mutual feeding is easily identified when a strain's number occurs in both the Feeds and Fed by columns for a particular strain (as for #4 and #7 below).

Feeds	Strain	Fed by
2, 4, 5, 7	1	3, 6
4, 5, 7	2	1, 3, 6
All	3	None
5, 7	4	1, 2, 3, 6, 7
None	5	All
1, 2, 4, 5, 7	6	3
4, 5	7	1, 2, 3, 4, 6

GUIDELINES FOR CONSTRUCTING A PATHWAY

1. Each intermediate substance is designated by an upper case letter (A, B, C, etc.).

2. Each mutant is designated by a number.

3. Every known step (reaction) in the pathway is shown by an arrow.

    •   A straight arrow between two intermediate substances means direct conversion of one to the other:

        A ———▶ B   A ——|—▶ B
                                  1

    A short line drawn across the arrow indicates a block of that mutant strain. The example here shows A cannot be converted to B in mutant strain 1.

- A split diverging arrow shows one substance is separated into two intermediates:
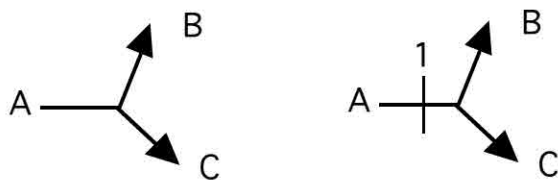
You should NOT draw lines across the split portions of the arrows as this can be misinterpreted as if there are two blocks in this pathway.

- A split converging arrow shows two substances are combined into one:

As mentioned above you should not draw line across the split portion of the arrow.

Each reaction is catalyzed by an enzyme which is designated by the number of the strain in which it is not functional. Every arrow should have a strain number associated with it.

DATA SET 1

Strain Being Fed

		1	2	3	4	5	6	7
	1	■	+	−	+	+	−	+
	2	−	■	−	+	+	−	+
Feeder	3	+	+	■	+	+	+	+
Strain	4	−	−	−	■	+	−	+
	5	−	−	−	−	■	−	−
	6	+	+	−	+	+	■	+
	7	−	−	−	+	+	−	■

DATA SET 2

Strain Being Fed

Feeder Strain		1	2	3	4	5	6	7
	1	■	+	+	+	−	−	+
	2	−	■	+	−	−	−	−
	3	−	−	■	−	−	−	−
	4	+	+	+	■	−	−	+
	5	+	+	+	+	■	+	+
	6	+	+	+	+	−	■	+
	7	+	+	+	+	−	−	■

If time permits& ..

INTRODUCTION TO THE FRESHWATER ECOLOGY LAB.

Today we are going to introduce the concepts and methodology of the Freshwater ecology labs (Chapter 10). You will need to form groups and run a hands-on ecological experiment to determine whether eutrophication is affected by chemical run-off.

At the end of the semester your group will need to turn in a lab report (writing assignment). Details about this assignment will be discussed in the upcoming labs.

Please read Chapter 10 to familiarize yourself with the laboratory procedure. Also read pages 1231-1243 in Biology (Campbell et al., 2008). You will be provided various fertilizers or your group can bring a chemical that you wish to test.

If you decide to bring your own chemical/nutrient (detergent, soap, fertilizer, medicine, etc.), it must be approved by Dr. Sarvary at least two days before the experiment.

BioG 1108

Name:_____

Lab Instructor:_____

BIOCHEMICAL PATHWAYS WORKSHEET

1. With reference to the syntrophic feeding trial data shown in Table 1, use conventions shown in the text to construct a pathway that is compatible with these data. Based on your pathway, complete Table 2.

Table 1. Syntrophic feeding trial relationships of six auxotroph strains in a hypothetical biochemical pathway.

Strain Being Fed

	1	2	3	4	5	6
1	■	+	+	+	+	+
2	–	■	+	–	–	+
3	–	+	■	–	–	–
4	–	+	+	■	–	+
5	–	+	+	+	■	+
6	–	+	+	–	–	■

(left labels: **Feeder Strain**)

PATHWAY:

Table 2. Important features of the auxotroph strains in the pathway shown above.

Auxotroph Strain	Substance(s) secreted due to block	Substance(s) missing due to block*	Strain(s) that it feeds	Strain(s) that feed it
1				
2				
3				
4				
5				
6				

* You need not list "end product".

2. With reference to the pathway shown below, complete Table 3 and 4 showing the expected syntrophic feeding relationships among the strains.

PATHWAY:
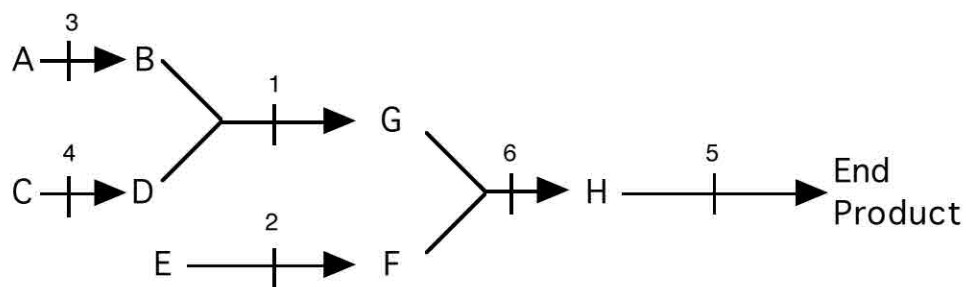
Table 3. Syntrophic feeding trial relationships of six auxotroph strains in a hypothetical biochemical pathway.

Table 4. Important features of the auxotroph strains in the pathway shown above.

Auxotroph Strain	Substance(s) secreted due to block	Substance(s) missing due to block*	Strain(s) that it feeds	Strain(s) that feed it
1				
2				
3				
4				
5				
6				

* You need not list "end product".

CHAPTER 4 –RECOMBINANT DNA TECHNOLOGY AND BIOINFORMATICS

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Revised by Mark A. Sarvary, June 2010

Laboratory Synopsis

The purpose of this three-day lab sequence is to learn a few commonly used molecular techniques, apply them to DNA sequencing, and ultimately search for nucleotide similarity between the study sequence and other similar ones deposited in databases using computer software. On the first day you will perform a ligation experiment on a plasmid and a foreign DNA fragment restricted by the same endonucleases. You will then conduct a transformation procedure to incorporate the ligated plasmid into the bacterial host, grow the bacterium and spread the culture onto agar plates containing an antibiotic. During day two you will examine the results of transformation, select bacterial colonies for plasmid purification and conduct an electrophoresis to examine the inserted sequence. On day three you will receive the picture from the gel you ran on day two and construct a standard curve to estimate the size of the inserted fragment. You will also discuss molecular conformation of plasmids under different conditions. In addition, you can learn the concept of DNA sequencing and perform sequence similarity search online from printouts of an automated sequencer.

LABORATORY OBJECTIVES

Conceptual

1.   Understand what plasmids are and how they are used as vectors for inserting genes into bacteria.

2.   Understand how restriction endonucleases are used to create recombinant DNA molecules and how these enzymes are named.

3.   Understand the importance of methylase enzymes.

4.   Understand some of the factors influencing transformation and explain a possible mechanism allowing foreign DNA to enter cells.

5.   Understand how electrophoresis can be used to separate DNA molecules of different sizes, the differences between agarose and polyacrylamide gel electrophoresis, and how the resolution of electrophoresis gels can be adjusted.

6.   Understand how to estimate the sizes of DNA molecules separated by electrophoresis.

7.   Understand how selection plating can determine if a new gene has been inserted into a host cell.

8.   Understand the mechanism of screening and how gene expression allows one to learn the success of DNA recombination.

---

*Martha Lyon provided invaluable help in the development and modification of this laboratory protocol.

Procedural

1.   Use micropipettes, microcentrifuges, and electrophoresis equipment.

2.   Use sterile techniques to manipulate bacteria.

3.   Construct a standard curve from size standards run in an electrophoresis gel.

4.   Estimate the sizes of unknown DNA fragments using a standard curve.

5.   Construct a restriction map based on DNA fragment data from a gel.

6.   Predict restriction fragment sizes given a restriction map.

7.   Interpret the results of plating in screening and selection.

8.   Utilize online resources to search DNA sequence similarity.

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

Lab One

1.   What is a genomic library?

2.   Why are restriction endonucleases that produce "sticky-ended" DNA fragments more useful in recombinant DNA studies than those enzymes producing "blunt-ended" DNA fragments?

3.   What is the purpose of subjecting the mixture of cut plasmid and the foreign fragment to 65°C treatment?

4.   To insert a foreign gene into a plasmid, frequently two different restriction endonucleases are used instead of one. Why?

5.   What is the probable function of "heat shocking" the cells during the transformation process?

6.   What is the purpose of the 45 minute incubation period immediately after "heat shocking" the cells?

7.   What is the function of the wild type *E. coli* control plates during selection plating with antibiotics?

Lab Two

1.   What are the phenotypes you will observe among the cells transformed with the ligated plasmids produced during lab one?

2.   What physical feature of DNA molecules determines the direction they will move in an electrical field?

3.   What is the relationship between DNA fragment size and migration distance in an electrophoresis gel?

4.   During the electrophoresis part of this study, what is the function of the lanes with DNA from the bacteriophage λ digested with *Hin*dIII?

5.   Name one advantage and one disadvantage in using ethidium bromide for staining DNA bands on an electrophoresis gel?

Lab Three

1.   How does the dideoxy DNA sequencing technique (Frederick Sanger's method) work? Is it the template sequence or the complementary sequence that is terminated prematurely? Is it the template sequence or the complementary sequence that is read out on the gel?

2.   Compared to Sanger's DNA sequencing method, what labeling technique makes it possible to mix the four sequencing reactions and perform sequencing using a piece of automated instrument?

3.   What is the tool BLAST designed for?

4.   What are the three possible conformations of a plasmid? Given the same run time in electrophoresis how far do they migrate in relation to one another in a gel?

READING ASSIGNMENTS

Day 1: from INTRODUCTION to Gel Electrophoresis and the section of PROCEDURE – Day 1
Day 2: from Gel Electrophoresis to DNA Sequencing & and the section PROCEDURE – Day 2
Day 3: from DNA sequencing & to PROCEDURE – DAY 1 and the section PROCEDURE – Day 3

HOMEWORK ASSIGNMENT

Complete the worksheet at the end of the chapter and submit it to your lab instructor by the specified deadline.

INTRODUCTION

Since DNA sequencing techniques were first introduced into the scientific community a few decades ago there have been many efforts put in place to decode the entire genomic sequences of various organisms. Because of the universal nature of the genetic code, nucleotide sequences have been recognized to provide insight into and have applications for evolutionary history, genetic mechanisms, protein function, medical diagnosis, and many other uses in biological science. Before the large-scale genome projects, such as the Human Genome Project, were launched, sequencing was conducted in many species with smaller genome sizes such as those of viruses and bacteria. With more efficient technology, multi-institutional collaboration, and the recognition of potential impact of genomic sequencing, recent efforts have established a governmental consortium to sequence the genome of close to 200 chosen species of eukaryotes. At the same time the genomes of about 100 additional species with larger genomes are being made into genomic libraries (see below) in the US for future sequencing efforts. In order to prepare for this endeavor, it is essential to be equipped with knowledge in genomic structure and skills in molecular biology techniques. For the next three days we are going to simulate some of the procedures involved in studying the genomics of an organism. We are going to produce a large quantity of identical (cloned) sequences of DNA, estimate the size in base pairs of the cloned fragment, and understand how DNA sequencing of the cloned fragment is conducted as a mental exercise. After receiving the results from a hypothetical source, we will search the sequence from databases available online to eventually attempt to decipher the possible function of these sequences.

Development of Genomic Sequencing Technology

In order to properly set up a procedure to decode the nucleotide sequence of an organism, it requires a few techniques and materials. Some of the molecular techniques and their purposes are listed below.

- To divide a large genome into smaller segments and store them safely – building a genomic library
- To produce large quantity of identical DNA fragment – cloning, or polymerase chain reaction (not discussed here)
- To check the size of the fragments and separate fragments from a mixture – electrophoresis
- To check the success of recombination, the joining of DNA of two or more sources into one molecule – screening
- To check the success of transformation – selection
- To cut and rejoin DNA fragments – requiring restriction endonucleases and ligase

Genomic Libraries

As mentioned earlier in the Introduction, efforts are being made in creating genomic libraries for various species in preparation for genomic sequencing. A **genomic library** is a collection of DNA fragments that together represent the entire genome of an organism. These fragments have been cloned and are stored in a stable condition (Figure 4.1). The practical reason for dividing up the genome is because the genome of most of the species is too large for sequencing in one single procedure. The materials used for packaging, storage and cloning vary in size and source. Because these materials are used as a vehicle carrying the DNA for sequencing they are called **cloning vectors**. In recent years, laboratories have favored using bacterial artificial chromosomes (BAC) derived from one special type of plasmid (extrachromosomal bacterial DNA) as cloning vectors to store genetic information from species with large genome sizes. The sizes of fragments stored in BACs are large, usually more than 100 kilobase long. After completing the packaging of the entire genome into individual BACs, the collection of sequences is like the book repository in a library; each "book", or clone, represents only one portion of the information stored in the library. Recombinant DNA technology, a collection of techniques that involve combining nucleotides of two or more different sources, lies at the heart of genome projects, and it often relies heavily on bacterial manipulation.

Genomic DNA cleaved with restriction enzymes

Genomic library

Plasmids introduced into bacteria

DNA fragments inserted into plasmids

Recombinant DNA molecules

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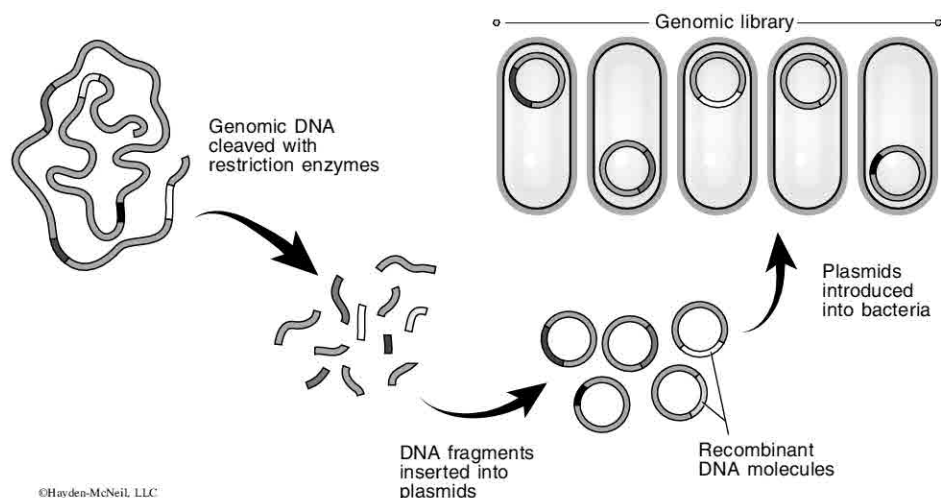
Figure 4.1. Schematic depiction of creating a genomic library from an organism. The plasmids are used to package the DNA fragments that will be sequenced.

Bacterial Genetics

Bacteria have been key organisms in advancing our understanding of the molecular basis of inheritance and in the development of recombinant DNA technology. Like the fungi, bacteria are easy to maintain in the laboratory. Also, bacteria have a very high reproductive rate. For example, the populations of some bacteria can double in size every 20–30 minutes. Because genetic analysis depends on the identification and isolation of rare mutations, working with an organism that can produce a large population rapidly is important. A single milliliter of bacterial culture can contain 10^9 cells. Even if a mutation occurs only once in a million cells, 1000 of the cells in one milliliter of culture would have the mutation.

Another advantage of bacteria is their genetic simplicity. Bacteria are prokaryotic organisms. They have a single, circular chromosome, which, lacking a nuclear membrane, resides directly within the cytoplasm. This chromosome is a single, large DNA molecule which, unlike the chromosomes of eukaryotic organisms, has no histone proteins associated with it. As in eukaryotes, genes are arranged along the length of the chromosome in a linear fashion.

Of all the bacteria, *Escherichia coli*, a normal inhabitant of the human large intestine, is certainly the best known and has been most important in the development of recombinant DNA technology. The genome of *E. coli* consists of about five million pairs of nucleotides and is about 1/600th the size of the human genome. The entire *E. coli* genome has now been sequenced. In this study, you will be using *E. coli* to gain experience with some of the methods used in recombinant DNA technology.

Plasmids

In addition to chromosomal DNA, bacteria often have extrachromosomal DNA called plasmids. The origin of plasmids is not completely clear. It is suggested they could have come from endosymbiotic process of foreign DNA or could have been from a spin-off of a section of chromosomal DNA from the bacteria. Just like chromosomal DNA, plasmids are circular. Because they do not have homologous sequences that code for similar functions, plasmid DNA and chromosomal DNA are both considered haploid. Plasmids are usually small in size, ranging from a few kilobases to a few hundred kilobases.

The **R-plasmids** (R for resistance) are an important class of plasmids carrying genes that code for enzymes that can modify antibiotics. The genes conferring resistance to kanamycin and ampicillin code for proteins that inactivate the antibiotics. A cell with a plasmid bearing an antibiotic-resistance gene will be able to grow in the presence of the antibiotic, whereas cells without the plasmid either will not grow or will die. Antibiotics can be useful as selective agents for determining which cells in a population possess plasmids with the appropriate antibiotic-resistance gene. If a foreign gene is inserted into an R-plasmid and a population of cells are exposed to the plasmid, treatment with the appropriate antibiotic will identify the cells that have picked up the plasmid and its foreign gene because only the cells with the plasmid will grow and be identified. Cells without the plasmid will be killed by the antibiotic. The process revealing cell colonies that have picked up the R-plasmids is known as **selection**.

Stringent plasmids replicate only when the bacterial chromosome replicates. This type of plasmid usually exists only as a single copy within the cell. **Relaxed plasmids** are able to replicate independently from the main chromosome and their copy number can range from 10 to 200 copies per cell. Foreign genes are **amplified** when carried on a relaxed plasmid. The plasmids used in this study are relaxed plasmids.

Before plasmids can be used as vectors they must be removed from their host bacterium and purified. The procedure of plasmid purification is called minipreparation or miniprep, a procedure we will not discuss here. Once the strain of bacteria containing the plasmid with the DNA insert of interest has been identified, we can grow the cells so that the plasmids they contain are in a sufficient quantity to be purified. The purified plasmids can be used for transforming other bacteria, or can be used for sequencing.

Restriction Endonucleases

Bacteria have evolved numerous mechanisms to help thwart the attacks of a group of viruses called **bacteriophages (phages** for short). **Restriction endonucleases**, or **restriction enzymes**, evolved in bacteria to destroy the phage before it can take over control of the cell. Each restriction enzyme recognizes and cuts (restricts) both strands of viral DNA at specific sites. These sites are characterized by being mostly four to six base pairs long. The enzyme binds at or near the recognition site and hydrolyzes the phosphodiester bonds of the double DNA helix (see Figure 4.5). After cleavage, the two exposed ends each have a phosphate group at the 5' end and a hydroxyl group at the 3' end of the DNA chain.

Table 4.1 shows several examples of restriction enzymes isolated from different bacteria. As you can see, some, such as *Hin*dII, cut straight across the DNA molecule to produce "blunt" ends, while others, such as *Hin*dIII, make staggered cuts resulting in "sticky" ends composed of unpaired nucleotide bases. Naming conventions for endonucleases are also illustrated by this table. The first letter of the name comes from the genus of the bacterium, the second and third letters from the specific epithet, the fourth letter, if present, is the strain, and the roman numeral indicates the position of this enzyme in the list of discovered enzymes for this species. So, *Hin*dIII (pronounced "*hin-dee-three*"), comes from *H* for *Haemophilus*, *in* for *influenzae*, d for strain d, and III indicates that this was the third endonuclease discovered for this species.

Table 4.1. Examples of restriction enzymes and their cleaving sequences; C = cytosine, G = guanine, T = thymine, A = adenine, Py = any pyrimidine base (C or T), Pu = any purine base (A or G). (modified from Michlos and Freyer 1990)

Species (strain)	Abbreviation	Sequence 5'–3' 3'–5'
Bacillus amyloliquefaciens H	*Bam*HI	G↓GATC C C CTAG↑G
Escherichia coli RY13	*Eco*RI	G↓AATT C C TTAA↑G
Haemophilus aegyptius	*Hae*II	Pu GCGC↓Py Py↑CGCGPu
Haemophilus aegyptius	*Hae*III	GG↓CC CC↑GG
Haemophilus influenzae Rd	*Hin*dII	GTPy↓PuAC CAPu↑PyTG
Haemophilus influenzae Rd	*Hin*dIII	A↓AGCTT T TCGA↑A
Providencia stuartii 164	*Pst*I	C TGCA↓G G↑ACGT C

Restriction enzymes are potent enzymes for protecting bacteria from virus attack, but what prevents these enzymes from destroying the bacterium's own chromosomal and plasmid DNA? For each restriction endonuclease produced by the bacterium, a companion enzyme called a **methylase** is also produced. Methylase adds a methyl group within the recognition sites of the endonuclease that occur on the bacterial DNA. The methyl group prevents binding of the endonuclease to recognition sites on bacterial DNA and the bacterial plasmid and chromosome are protected from restriction. For example, the restriction endonuclease *Eco*RI has a methylase enzyme called *Eco*RI methylase, that catalyzes the reaction adding a methyl group to the second adenine from the 5' end of the recognition site. This prevents binding of *Eco*RI to the site and restriction cannot occur. See Figure 4.2.

Cloning

To conduct a DNA sequencing procedure, the first requirement is to make a large quantity of identical copies of the DNA fragment of interest. This can be accomplished by inserting the foreign DNA into a relaxed plasmid and incorporating the plasmid into a host *E. coli* cell (**transformation**, see below). The plasmid then has become a cloning vector. Three structural components are needed for a plasmid to be used as a cloning vector: an origin of replication, a gene that produces an antibiotic-resistant substance, also called a selective marker, and a region with restriction sites containing one copy of each of many sequences recognized by various restriction enzymes. The region is called a multiple cloning site or polylinker region (Figure 4.3).

Unmethylated DNA

...G A A T T C...
...C T T A A G...

Recognition sequence

*Eco*RI enzyme
binds and cuts

...G A A T T C...
...C T T A A G...

A A T T C...
...G   G...
...C T T A A

DNA cut

Methylated DNA

...G A A T T C...
...C T T A A G...

Methyl group

*Eco*RI enzyme
cannot cut

...G A A T T C...
...C T T A A G...

DNA protected

©Hayden-McNeil, LLC
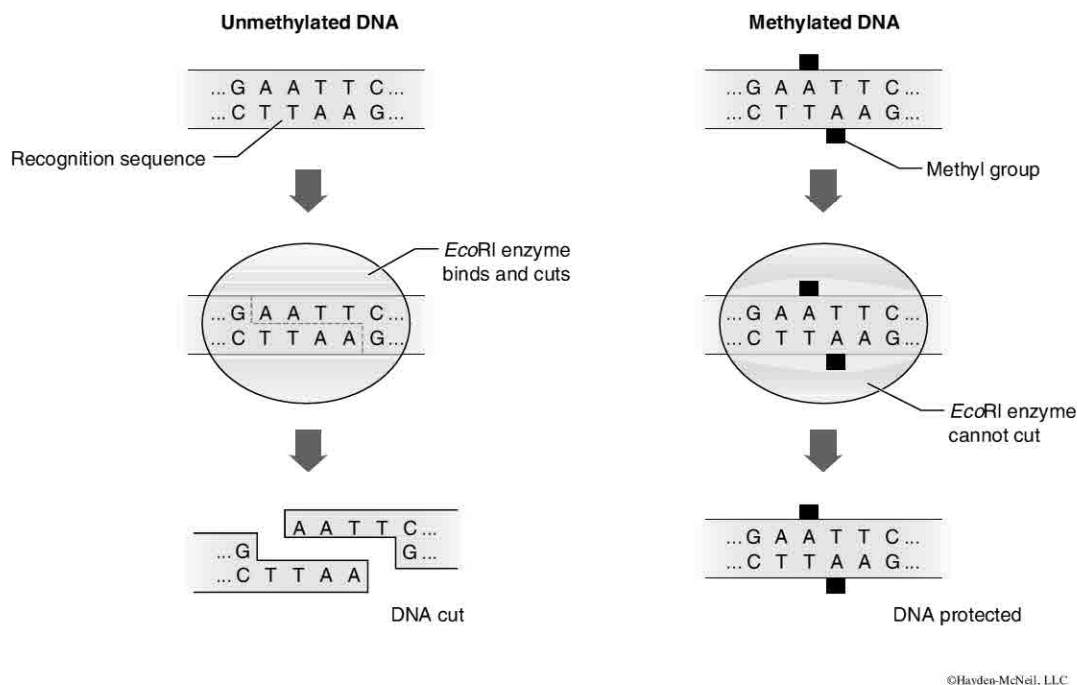
Figure 4.2. The *Eco*RI restriction-modification system. Left, shows binding of the *Eco*RI enzyme to the recognition site and subsequent cutting of DNA. Right, shows that the methyl groups added to the recognition site by *Eco*RI methylase prevent proper binding of *Eco*RI and protects the DNA from cutting.

When a foreign DNA is purified for the purpose of sequencing, it is cut by a restriction enzyme that creates numerous fragments with staggered ends. The same enzyme will be used to cut the plasmid used as the cloning vector. When the foreign DNA fragments are mixed with the linearized cloning vector, the hydrogen bonds between the complementary bases are paired up subsequently. If we use only one restriction enzyme, the two cut ends of the plasmid can self-anneal, preventing insertion of the foreign DNA. Therefore we should use two restriction enzymes so that only one end of the plasmid can pair up with one end of the foreign DNA fragment cut by the same restriction enzyme. Using two restriction enzymes can also ensure a preset orientation for the insert. After the reannealing (the hydrogen bond formed between complementary bases), the missing covalent bond at both ends of DNA strands will be sealed by adding the enzyme **ligase**.

pUC8, the plasmid you will be using in lab, contains an ampicillin resistance gene (*amp*^{r}). Additionally, the multiple cloning site lies within the *lac*Z gene that codes for the enzyme β-galactosidase (Figure 4.3). As you will see in lab, this is a useful feature because it allows one to use a simple biochemical assay to distinguish bacteria containing plasmids with insert of foreign DNA to be cloned from those without, a process called **screening**. In particular, bacteria containing plasmids with inserts will not synthesize β-galactosidase because the DNA fragment of interest has been inserted into the middle of the *lac*Z gene. Notice that there is only one *Eco*RI and one *Hin*dIII restriction sites in pUC8, and both sites are located within the *lac*Z gene. When the two restriction enzymes are used the only sites that will be cut are in the *lac*Z gene, and consequently the location for the inserted fragment. When the *lac*Z gene is transcribed, its mRNA is altered because of the inserted sequence, thus the gene is unable to be translated into a functional enzyme.
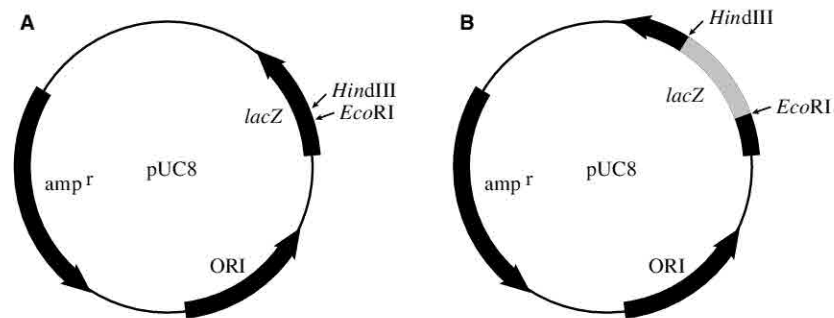
Figure 4.3. pUC8 with an ampicillin resistance gene (amp^r), a *lacZ* gene serving as the polylinker regions containing the *Eco*RI, *Hin*dIII and other restriction sites, and the origin of replication (ORI). A shows an intact pUC8; in B, a foreign sequence is inserted between *Eco*RI and *Hin*dIII sites. The insertion disrupts the *lacZ* gene, which cannot produce the enzyme β-galactosidase. The arrows indicate the direction of transcription for *lacZ* and ampicillin resistance genes and replication for ORI.

Given the information, how do we visualize and distinguish bacteria that contain plasmid from those without the inserted fragment? Recall from the lecture on prokaryote genetics, lactose can form the inducer to help the transcription of *lacZ* gene, but lactose will also be cleaved by the product of *lacZ* gene, β-galactosidase, which produces glucose and galactose. Because neither glucose nor galactose can be easily visualized, a good substrate candidate will be a substance that produces color (chromogenic) after being hydrolyzed by β-galactosidase. So instead of the natural substrate for β-galactosidase, we are plating the bacteria on a medium containing the lactose derivative X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside). After being cleaved by β-galactosidase, X-gal produces a blue precipitate that can be easily visualized as shown in the diagram below.
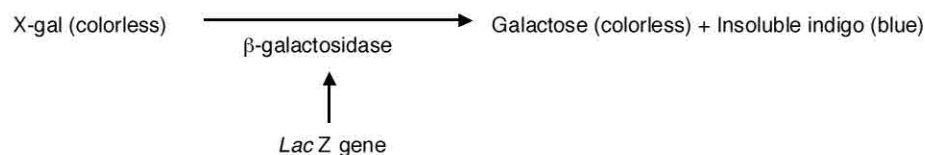
However, X-gal, being a substrate for β-galactosidase, is not able to induce the synthesis of the enzyme. In order to transcribe the *lacZ* gene we need one additional substance that can continuously induce the expression of *lacZ* gene without having itself being modified by the gene product. The synthetic molecule IPTG (isopropylthio-β-D-galactoside), another molecule that mimics lactose, is used as an inducer. IPTG cannot be hydrolyzed by β-galactosidase thus can induce the synthesis of β-galactosidase without interruption. Under the condition where the growth medium has sufficient amount of X-gal, β-galactosidase can cleave its bond and produce sufficient quantity of blue product in the colonies for visualization. In your experiment you will use a growth medium that contains nutrients with both X-gal and IPTG. As predicted by the design, the bacterial colonies containing plasmids with a functional *lacZ* gene, i.e., without the inserted fragment, will appear blue; the colonies of bacteria containing plasmids that have been successfully recombined with the insert will appear white.

Transformation

Bacteria are considered transformed when they have successfully acquired and are able to replicate the DNA vector. Transformation efficiency using plasmids is low; only a small fraction of bacteria successfully take up a plasmid. Consequently, it is necessary to distinguish transformed bacteria from those lacking the plasmid. This process, called **selection** as described earlier in the Plasmid section, is made possible by the presence of a drug-resistant gene in the plasmid (e.g., ampicillin resistance in pUC8). Successfully transformed bacteria will grow on a medium containing the drug while those lacking the plasmid will not. Selection assays also include a control; bacteria are grown on a plate lacking the selective agent to ensure that the bacteria are viable.

Collectively, selection and screening allow one to determine: 1) which bacterial colonies have the plasmid (selection) and 2) of those colonies with the plasmid, which have plasmids containing the insert (screening) and, thus, have cloned the DNA of interest. After the selection and screening process is completed, the bacterial strain that has the plasmid with the inserted fragment will be cloned and its plasmid purified.

Cells that can take in and express foreign DNA are called **competent**. The natural occurrence of competent cells in a population of bacteria is low; most are not capable of being transformed. Scientists have discovered conditions that increase the number of competent cells in a population and this information lends some insight into the mechanism whereby foreign DNA enters the cell.

The following conditions improve transformation:

1.   Use of cells from a population undergoing exponential growth. Bacterial population growth typically shows four phases, as depicted in Figure 4.4. The proportion of cells that are competent is maximum for a population in the exponential phase of growth when the population is growing very rapidly. The reason for this is unknown.

2.   Arresting of cell growth by transferring cells to low temperatures (0°C). This is typically done by keeping cells in an ice bath.

3.   While being maintained at low temperature (condition 2), treating the cells with divalent cations, such as Ca^{++} provided by a solution of $CaCl_2$.
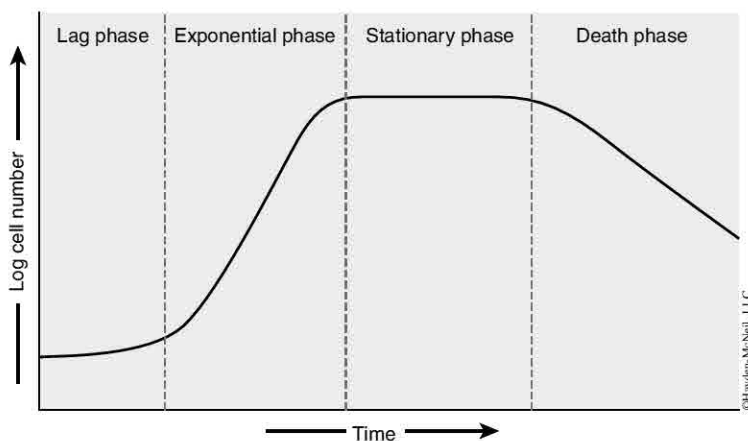
Figure 4.4. Typical growth curve for the bacterium *Escherichia coli*.

4.   **Heat shocking** cells by transferring cells directly from low temperature to 37–45°C.

It is hypothesized that foreign DNA such as plasmids are able to diffuse into a bacterial cell through pores called **adhesion zones**, where the inner and outer membrane of the cell are continuous. Normally, one would expect electrostatic repulsion between the negatively charged ends of phospholipids lining these pores and the negatively charged phosphate ions of the DNA molecule (see Figure 4.5) to prevent passage of DNA through the pore. Probably the treatment with a solution of divalent cations allows the positive cations to complex with the phosphate ions of the membrane phospholipids and the plasmid DNA thereby reducing the electrostatic interactions between the DNA and the pore. A recent study suggests that the heat shock treatment facilitates entry of plasmids by reducing membrane potential of the competent cells. The decrease of membrane potential lowers the level of negativity inside the cell, which further reduces the electrostatic repulsion, making the entry of negatively charged DNA molecules more feasible.

5.   DNA fragment size is another major factor that affects how readily foreign DNA will be incorporated into a cell. Large pieces of DNA or large plasmids will be less likely than smaller pieces of DNA or smaller plasmids to move into a cell.

Gel Electrophoresis

The application of gel electrophoresis for separating nucleic acids was also important in the development of recombinant DNA technology. Previously, DNA molecules of different sizes had to be separated by the cumbersome, expensive process of density-gradient centrifugation, as in the famous Meselson and Stahl experiment on ^{15}N labeled DNA. In 1970, Daniel Nathans used an electrical field to separate DNA fragments moving through a gel of polyacrylamide. Nathans had previously labeled the fragments with a radioactive isotope, so that after separation, he could locate the bands by letting the radioactivity expose a photographic plate placed on top of the gel. A further advancement in use of electrophoresis was the introduction of the fluorescent dye **ethidium bromide**. This dye binds to small amounts of DNA on the gel and fluoresces when exposed to ultraviolet light allowing the bands to be visualized directly on the gel. Ethidium bromide molecules intercalate between nucleotide base pairs in double stranded DNA. In doing so they can cause alterations (mutations) in the DNA.

Agarose Gel Electrophoresis

The essential features of electrophoresis are simple; an electrical field is established in a medium through which charged molecules can diffuse. Nucleic acids are acids because the phosphate groups of the sugar phosphate chains ionize (by loss of a H^+ from the oxygen) when in solution. The resulting negatively charged phosphate ions give nucleic acids a net negative charge. See Figure 4.5. As a result of their negative charge, nucleic acids migrate toward the positive pole in an electrical field.

In electrophoresis, migration of charged particles occurs through a gel composed of either agarose or polyacrylamide. Agarose, a gel made from purified agar, is good for relatively long pieces of DNA; polyacrylamide, a synthetic polymer, is better for small DNA molecules in the 2 to 500 nucleotide base pair range. Both gels act like molecular sieves. Larger molecules will move more slowly through the gel than smaller molecules. The density of the gel can be adjusted by varying the percent of the agarose or polyacrylamide in the solution. A denser gel has smaller spaces than a less dense gel. Consequently, fragments will move more rapidly in a loose gel and more slowly in a dense gel. Thus, the density of the gel determines the resolution of the gel. In this laboratory, you will make a 1.0% agarose gel. This will produce a gel density best for resolving the size range from a few hundred to thousands of base pairs of DNA fragments encountered in this study.

Agarose and polyacrylamide gels are made by pouring a solution of the material into a casting tray in which suspended is a comb with a variable number of teeth. After the gel has solidified, the comb is removed creating depressions or **wells** into which samples are placed. The gel is covered with a buffer solution. A power supply connects two electrodes on opposite ends of the gel. The end of the gel containing the DNA samples is next to the negative electrode (why?). The electrical field that is established within the gel causes the DNA to migrate.

Figure 4.5. Single-stranded nucleic acid chain. Circles show the negatively charged oxygen atoms that result from the loss of a proton.

Smaller molecules (fragments) move more rapidly through an electrophoresis gel than larger molecules and end up nearer the positive end of the gel. Molecules of the same size move at the same rate so electrophoresis separates populations of molecules of the same size into "bands" on the gel. Because DNA is colorless, you must add a dye, called a "loading dye" that will move as fast as the smallest fragments. When the dye reaches the positive end of the gel, the run is complete. The gel is then stained so the DNA bands can be seen. As mentioned earlier, ethidium bromide is a commonly used stain. This highly mutagenic substance binds to DNA by intercalating between the stacked bases of DNA, producing highly concentrated patches of the dye in the exact location of DNA bands on the gel. When the gel is photographed in UV light, the bands fluoresce and appear bright pink. (Ethidium bromide absorbs UV-irradiation in the 300 nm and 360 nm range and subsequently re-radiates this energy at 590 nm in the red-orange region of the visible spectrum.) Fluorescing bands are recorded on photographs as light areas on a dark background. Each band is a collection of millions of molecules of DNA of the same size. The intercalation of ethidium bromide between the nucleotide base pairs allows detection of as little as 1–10 ng of DNA. Since all bands in a single lane contain the same number of fragments, the intensity of staining by ethidium bromide is proportional to the size of the DNA molecules in the band. BECAUSE ETHIDUM BROMIDE IS A POTENTIAL MUTAGEN (CARCINOGIN), IT MUST BE HANDLED CAREFULLY WITH DISPOSABLE GLOVES.

Because smaller molecules move faster, you expect to see an inverse relationship between the distance moved from the sample well and the size of the DNA fragment. DNA size is usually expressed as the number of nucleotide base pairs. Specifically, if you plot the log of the migration distance against the log of the number of base pairs, a linear relationship will result. To "calibrate" this curve, one typically includes a sample producing bands whose fragment sizes are known. These **size standards** then allow you to construct a standard curve defining the relationship between the migration distances of DNA fragments and the log of fragment size in base pairs for the electrophoresis system being used. The standard curve can then be used to estimate the base pair lengths of the fragments in any bands observed from the unknowns on the gel.

In this lab cloned DNA fragment is retrieved by digesting the plasmid with the same restriction enzymes that were used during the original insertion. The relatively large pieces of plasmid DNA and the smaller, cloned fragment are then separated by electrophoresis on an agarose gel. The size of the inserted fragment is determined by comparing the distance this fragment migrated to a standard curve constructed from phage λ fragments produced by *Hin*dIII digestion and run on the gel simultaneously. The DNA is visualized by first incubating the gel in a solution of ethidium bromide and photographed when illuminated with UV light.

First Recombinant DNA Study

The first recombinant DNA study was conducted more than thirty years ago by Stanley Cohen, Annie Chang, Herbert Boyer, and R. B. Helling (Cohen and others 1973). Working with *E. coli*, they used the restriction enzyme *Eco*RI to isolate a gene conferring kanamycin-resistance. They used the same enzyme to open the plasmid pSC101, which already contained a gene for tetracycline-resistance. DNA ligase was used to form a new recombinant plasmid consisting of the pSC101 combined with the kanamycin-resistance gene. Transforming *E. coli* with this plasmid vector produced a new strain of bacteria that could grow on media to which both antibiotics had been added. When they isolated plasmids from the strain and cut up the plasmids with *Eco*RI, they observed two bands on the resulting gel after electrophoresis, one corresponding in molecular weight to the original pSC101 plasmid and the other to the piece with the kanamycin-resistance gene. Cohen, Chang, Boyer, and Helling were the first to use recombinant DNA technology to change the genetic constitution of a living organism. Their study consisted of the following steps, which today are six commonly used methods in DNA technology:

1. Ligation of fragments to make recombinant molecules.
2. Transformation of recipient cells with the plasmid vector.
3. Selection of transformants using antibiotic resistance.
4. Generation of DNA fragments with restriction endonucleases.
5. Separation and analysis of DNA fragments with gel electrophoresis.
6. Isolation and analysis of plasmid DNA from the transformed cells.

Cohen and others have opened the door for many other molecular biologists and geneticists who use and modify their procedures for purposes that go far beyond those of the original study. In this lab we will use most of their experiments with modifications for the purpose of DNA sequencing (see Figure 4.6 for day one procedures). The actual hands-on procedure for you will include steps 1 through 3 and step 5 above. Step 4 will be done for you by the staff members; step 6 will be included in a discussion. In addition we will conduct a mental exercise of DNA sequencing using Frederick Sanger's method, a procedure often done commercially using an automated sequencer. Ultimately we will receive sequence printouts and will use them to search for sequence similarity through an online search tool BLAST.
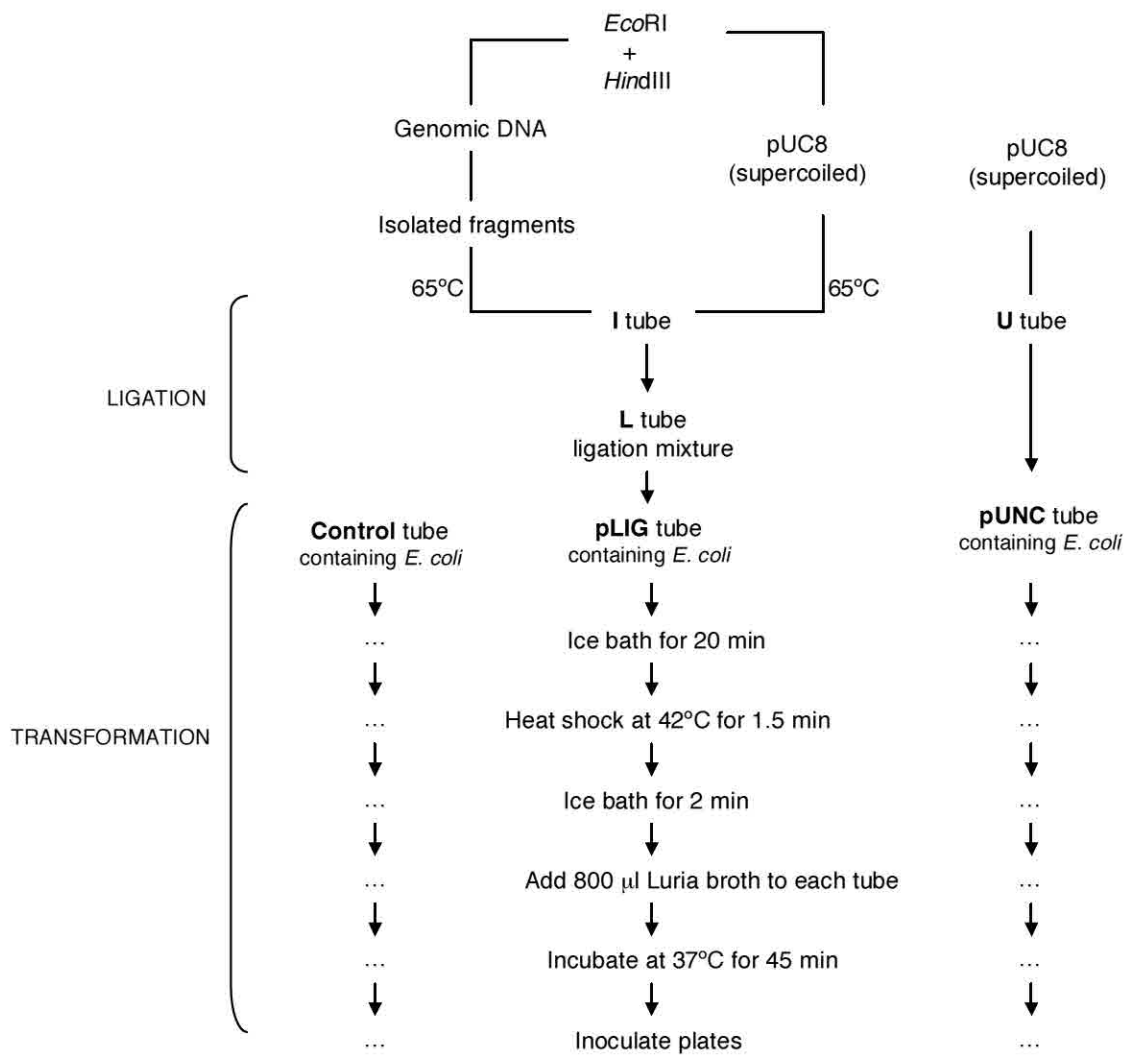
Figure 4.6. Flow diagram for procedures in lab one.

DNA Sequencing Using Sanger Method

The sequence of the DNA insert can be analyzed using the purified plasmids collected from the white colonies grown on the medium with ampicillin, X-gal and IPTG. The automated sequencing method commonly used today is based on the concept and technique created by Frederick Sanger and his colleagues in the 1970s. The key ingredient of this method is dideoxynucleoside triphosphates (ddNTPs) that are added to in vitro DNA synthesis to terminate the reactions because they lack of the 3' hydroxyl group for the next nucleotide to attach. The method has four separate DNA polymerization reactions taking place in individual reaction tubes. Among the four reaction tubes the common ingredients are the DNA template to be sequenced, the primer to start the polymerization, four types of dNTPs as the building blocks of the new sequences, and the DNA polymerase to make the reaction occur. The primer also has a radioactive label on its 5' end for visualization later in electrophoresis. In addition to the common ingredients, each of the four reaction tubes contains a unique ddNTP (ddATP, ddTTP, ddCTP, or ddGTP) in a lower concentration than that of the ddNTPs contained in each reaction tube. When the new DNA strand is being synthesized, most often an appropriate dNTPs will be added to the growing DNA strand complementary to the nucleotide of the template and allow the new DNA strand to elongate. When a ddNTP is incorporated, however, to the growing strand the elongation will be terminated as described above. Given enough cycles of synthesis, complementary DNA strands of various lengths will be produced, each of which carries a ddNTP at its the 3' to indicate the last nucleotide of that DNA strand. After the reaction samples are run side by side on a gel. The fragments separated by their size are then detected by autoradiography. Because the synthesized strand is complementary to the template sequence, the sequence of the complementary strand can be read directly from the autoradiogram in the order from the smallest to the largest fragment (Figure 4.7).
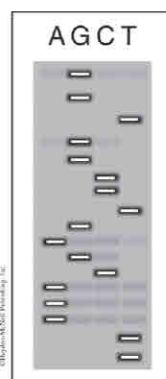
Figure 4.7. A partial autoradiogram showing the four reactions of the dideoxy sequencing method. Each lane is labeled for which one of the four ddNTPs was added in the reaction. The sequence is read from the bottom of the gel as the smallest fragment carrying the first terminated nucleotide travels the fastest. The next smallest fragment reflects the second base of the sequence. The sequence is read as "TTAAACGAGTCCGGTGG".

Since the dideoxy method was invented new visualization methods have been developed to allow automation of the DNA sequencing process. The new visualization methods include a few modifications. First, instead of a radioactive label, a fluorescent tag is used. The fluorescent label can be tagged either on the primer or on the ddNTPs. If the primer is labeled, the four reactions will be essentially the same as the original Sanger Method, in which four reactions occur in separate tubes and later loaded in separate lanes in gel electrophoresis. If, however, each of the four types of ddNTPs is labeled with a unique fluorescent dye (A, green; T, red; C, blue and G, yellow) the reaction can take place in one tube. the color-coded DNA fragments will run through a capillary tube where laser beam excites the dye, which causes fluorescence. A detector senses the flashes of light and transmits the data to the computer. In principle one can read out a lane of sample and determine the sequence of the fragment based on the

color of the dye on the fragment. However, without the need for one to read off the gel, the computer processes the signals and generates colored peaks in the order of the nucleotides of the sample in question (Figure 4.8). Illustrations of the automated sequencing procedure can be found in Figure 20.12 in Campbell et al., 2008.
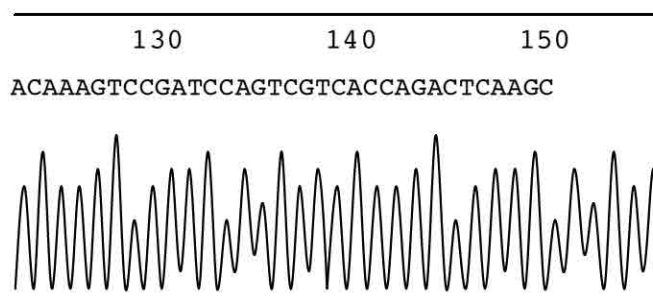
Figure 4.8. A partial electropherogram from the automated sequencer printout. Each of the peaks shows one of the four possible colors corresponding to the nucleotide printed above the peak. The numbers at top indicate the number of the nucleotides in the sequence.

Analysis of DNA Sequence Similarity Using BLAST

In the last two decades there has been an exponential growth of genetic information obtained through DNA sequencing. In order to compare and share information biologists and information technologists have established nucleotide and protein sequence databases primarily in the US, Europe and Japan to serve the purpose of information exchange, and the discipline of **bioinformatics** that combines biology and information technology is created. With so much information how does a biologist who has sequenced an unknown gene compare this result with the published data? An Internet search tool BLAST (Basic Local Alignment Search Tool) developed in the 1990s allows one to enter a sequence in questions and compare it with those in the databases. There are two parameters used to assess the level of similarity, the "Score" and the "E value". The "Score" indicates the similarity between the sequence in question and the sequences in the databases, the higher the score the better the match. The "E value" indicates the probability of a random match between the two sequences being compared, the lower the value (e.g., e^{-67}), the less likely that the two sequences are a random match. Based on these two parameters one can determine if the sequence in question is closely related a gene or a protein.

PROCEDURES - LAB ONE

Ligation of pUC8 with Foreign Genomic DNA fragment

This procedure involves transferring content of I tube to L tube (see below). You will be using a 2–20 µl micropipettor to set up the ligation mixture. To obtain practice with this instrument, complete the exercise on dispensing small volumes with the 2–20 µl micropipettor. Practice with Digital Micropipettors

❑ Read the section of the Appendix 1. that describes the use of digital micropipettors and complete the exercise on dispensing small volumes with the 2–20 µl micropipettor.

❑ Receive tube labeled "I" (which stands for insert) from your lab instructor. The tube contains digested pUC8 plus the isolated genomic fragment for cloning. *Eco*RI and *Hin*dIII activity in this tube has been destroyed by heating to 65°C in a water bath.

❑ Obtain a 1.5 ml microcentrifuge tube labeled "L" from your instructor. This tube contains 10 µl buffered ligase with ATP. DNA ligase and ATP promote ligation of the fragments created by *Eco*RI and *Hin*dIII. Keep tube L on ice.

❑ Set a 2–20 µl micropipettor to 10 µl. Use the procedure you practiced to fill the micropipettor and add 10 µl of content from I tube to L tube.

❑ Close the cap of the microcentrifuge tube and mix its contents by gently tapping the bottom of the tube with your finger

❑ Place tube L into a centrifuge opposite a balance tube. Spin for several seconds to pool and further mix the chemicals.

❑ Incubate tube L at room temperature for 10 minutes.

❑ Continue with **transformation**.

Transformation of *E. coli* with Uncut and Ligated pUC8 Plasmids

❑ Clear all books, papers, etc. from the workspace and disinfect the area with alcohol. All of the procedures you perform in this part of the study must be done under sterile conditions.

❑ Each group will receive a "U" (meaning uncut) tube that contains uncut, supercoiled pUC8. In addition, your group will need the following equipment:

- 700 µl of competent, wild type *E. coli* in an microcentrifuge tube
- 3 ml sterile Luria–Bertani broth (LB) in a culture tube
- 3 – sterile culture tubes
- digital micropipettors, 2–20 µl and 20–200 µl
- tips for micropipettors, sterilized
- 250 ml beaker filled with ice and some water
- 1 marking pen
- 1 stopwatch (one group member exchange ID card)

- 9 agar plates - 3 each with no stripe (LB only), red stripe (LB + ampicillin), and red + black stripes (LB + ampicillin + X-gal + IPTG)

- alcohol, spreading rod, glass petri dish, and alcohol lamp

❑ Apply pieces of masking tape to three sterile culture tubes and label them pLIG, pUNC and control. Chill the tubes on ice for at least two minutes.

❑ Use the following procedure to transfer 200 µl of competent *E. coli* cells into each tube. Note: these cells are suspended in a CaCl$_2$ solution so that the Ca^{++} ions will neutralize the phosphate ions of the phospholipids in the cell membrane.

   √   Set the 20–200 micropipettor to 200 µl. Insert a clean tip onto the micropipettor.
   √   Remove the cap from the tube with the competent cells.
   √   Fill and expel the micropipettor twice to insure that the culture is thoroughly mixed. Now, remove 200 µl of culture.
   √   Recap the culture tube.
   √   Quickly remove the cap of the pLIG tube.
   √   Insert the pipette tip and expel the culture into the pLIG tube.
   √   Recap and place the pLIG tube into a beaker with ice.
   √   Repeat these steps to add 200 µl of competent cells to the pUNC tube and the control tube.

❑ Use the 2–20 µl micropipettor set to 10 µl to transfer 10 µl ligated pUC8 from tube L (prepared in the Ligation step above) to tube **pLIG**. Be certain to put the ligation mixture directly into the culture in the bottom of the tube. Mix the contents of the **pLIG** tube by tapping lightly on the bottom of the tube.

❑ Similarly, transfer 10 µl of uncut pUC8 from U tube to the culture tube labeled **pUNC**.

❑ Return all tubes to the beaker of ice for 20 minutes. Incubation at low *temperatures helps to further stabilize the charges on the cell membrane. Ca^{++} ions from the CaCl$_2$ neutralize the negative charges on the plasmid DNA phosphate groups.*

❑ Heat shock cells by <u>rapidly</u> transferring the tubes **pLIG, pUNC,** and **control** from the ice to a 42°C water bath. Heat shocking facilitates movement of plasmids into the cell.

❑ Remove the tubes from the water bath after 1.5 minutes and return them to the ice bath for 2 minutes.

❑ Be sure that the 20–200 micropipettor is set to 200 µl and has a clean tip. Transfer four 200 µl aliquots for a total of 800 µl of Luria broth to the **pLIG** tube.

❑ Eject the used tip and insert a clean tip. Transfer four 200 µl aliquots for a total of 800 µl of Luria broth to the **pUNC** tube.

❑ Eject the used tip and insert a clean tip. Transfer four 200 µl aliquots for a total of 800 µl of Luria broth to the **control** tube.

❑ Incubate tubes **pLIG, pUNC,** and **control** in a 37°C shaking water bath for 45 minutes. Since pUC8 is a relaxed plasmid, during incubation the plasmid replicates producing many copies per cell and amplifying their antibiotic-resistance gene.

Spreading Plates

In the following procedure, there is a real danger of fire. To minimize this danger arrange your equipment on the work area so that the alcohol dish is well removed from the alcohol burner and flaming spreading rod, as shown in Figure 4.9. Be sure to immediately replace the lid on the alcohol dish each time it is used.
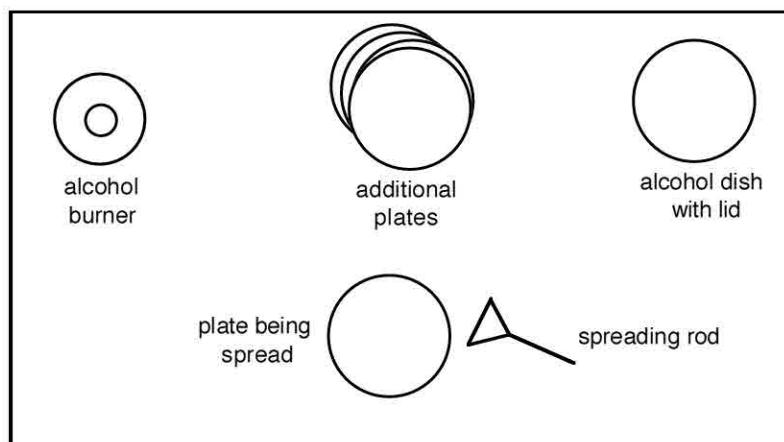
Figure 4.9. Suggested spatial arrangement of major materials used for plate spreading.

An assembly-line approach should be used to set up the plates, with different group members responsible for adding the cell suspensions and spreading the suspensions on the agar surface. Note: as cell suspension is added to each plate it must be immediately spread so teamwork is essential. Sterile technique is required in setting up these plates.

Table 4.2. Organization of plates and volume of transformed cells spread on each plate. Each type of transformation cells will be spread on three types of plates indicated. The abbreviations are as follows: LB: Luria-Bertani Broth, A: ampicillin, X: X-gal, and I: IPTG.

		Plate type		
		LB only (no stripe)	LB+A (red stripe)	LB+A+X+I (red+black stripes)
Transformation cell type	C (control)	100 µl	100 µl	100 µl
	pUNC	100 µl	100 µl	100 µl
	pLIG	100 µl	100 µl	100 µl

❑   Label the three **no stripe** plates (LB only) **pLIG**, **pUNC**, and **C.**

❑   Label the three **red stripe** plates (LB + ampicillin) **pLIG**, **pUNC**, and **C.**

❑   Label the three **red-and-black stripe** plates (LB + ampicillin + X-gal + IPTG) **pLIG**, **pUNC**, and **C.**
❑   **IMPORTANT**: The bacteria in the **pLIG, pUNC,** and **control** tubes have settled. Push down firmly on the cap of the culture tubes to be sure that they are tightly closed. Now, invert the tubes twice to obtain a uniform suspension before removing bacteria in the next two steps.

❑   Organize the plates so that three plates of the same label are in one stack (see Table 4.2). Set the 20–200 µl micropipettor to 100 µl and insert a clean tip. Transfer 100 µl from tube **C** to the center of each plate labeled **C**. Time your transfers so that each aliquot can be spread immediately after it is placed on the plate.

❑   Dip the spreading rod into a petri dish with alcohol. Note: immediately replace the lid on the petri dish containing the alcohol.

❑   Pass the rod through the flame of your alcohol lamp to ignite the alcohol.

❑   Remove the rod from the alcohol flame and allow the alcohol to burn off. Note: hold the spreader at a downward angle to prevent flaming alcohol from running on to your hand.

❑   Lift the lid of a petri dish only enough to allow insertion of the spreading rod. Cool the rod by touching it to the inside lid of the plate.

❑   When the rod is cool, spread the cells by moving the spreading rod back and forth across the agar surface several times.

❑   Rotate the plate 1/4 turn and repeat the spreading motion.

❑   Continue with the procedure of dipping the rod into alcohol and flaming it until all three plates are spread.

❑   Insert a clean tip on to the 20–200 µl micropipettor. Transfer 100 µl from tube **pUNC** to the center of each plate labeled **pUNC**. Repeat the transferring and spreading procedure until all three plates are treated.

❑   Repeat the same procedure by inserting a clean tip on to the 20–200 µl micropipettor. Transfer 100 µl from tube **pLIG** to the center of each plate labeled **pLIG**. Repeat the transferring and spreading procedure until all three plates are treated.

❑   Tape your plates together in stacks of four or five and invert the stacks. Label your names, lab time and lab room number on the tape. The plates will be placed in a 37° incubator for you for at least 12 hours. The plates will then be removed from the incubator and refrigerated until next laboratory session.

PROCEDURES - LAB TWO

Counting Colonies

After you receive the plates your group inoculated last lab, you will determine the number and color of colonies that grew on the antibiotic-containing plates. Each colony you observe is a clone of identical cells that resulted from a bacterial cell that was successfully transformed with a plasmid containing the antibiotic-resistance gene. In addition, the color of the surviving colonies is determined by whether a functional *lac*Z gene is present in the plasmid. Recall from the introduction section of the chapter, in the presence of the chromogenic substrate X-gal and the inducer IPTG, a functional *lac*Z gene can produce β-galactosidase to hydrolyze X-gal and produce a blue precipitate in the colonies.

❏ Observe the three plates that contained no antibiotics in the agar (plates without stripes). Compare the surface of these plates with the surface of the six plates containing antibiotics.

If the number of bacterial cells that are growing on a plate is great, then the colonies that they produce will converge to form a solid "lawn" of bacteria. Do you observe a bacterial lawn on the three plates that contained no antibiotics? If the number of bacterial cells that are growing on a plate is smaller, then the colonies that they produce will not converge and distinct, non-overlapping cell masses will result.

? Why are distinct colonies seen on the antibiotic-containing plates instead of a lawn of cells? What happened to most of the cells that produced the lawn on the plates without antibiotics? Recall that all plates initially received about the same number of cells.

? In this study, what is the purpose of the plates containing no antibiotic?

❏ Divide any of the remaining six plates with colonies present among the group members.

❏ Turn the plate upside down and remove the masking tape label. Use a marking pen to mark colonies as they are counted. If there are more than 50 colonies per plate, you may wish to divide the plate into four quarters with a marking pen and count colonies in each quarter separately.

❏ Enter your group's data in Table 4.3.

? In this study, what is the purpose of the plates that received wild type *E. coli* (the plates labeled **C**)? Explain.

? What is the purpose of subjecting ampicillin to wild type cells transformed with either plasmid without an insert and the cells transformed with the plasmid containing the insert?

Table 4.3. The number and color of colonies resulting from wild type *E. coli* treated with recombinant pUC8 plasmids containing a foreign fragment, pUC8 plasmids without any insert, or no plasmid growing on plates containing either no antibiotic, with ampicillin, or with ampicillin, X-gal and IPTG.

Type of plate medium

		LB only	LB + ampicillin	LB + ampicillin + X-gal + IPTG
Transformation cell type	Wild type (C)			
	Wild type + plasmid without insert (pUNC)			
	Wild type + plasmid with insert (pLIG)			

Transformation efficiency is determined by the following equation.

Transformation efficiency = $\dfrac{\text{\# of Transformed cells}}{\text{Total plasmid DNA spread on plate}}$

1. Let us assume that the plasmid DNA concentration was 0.01 µg/µl. You added 10 µl, giving a total of **0.1 µg** of plasmid DNA.
2. You added the plasmids to 200 µl of competent cells for transformation followed by 800 µl of recovery LB solution, a total of 1000 µl.
3. You took out 100 µl of the total transformants for spreading on each plate. This is **1/10** of the total volume in the culture tube.
4. The amount of DNA plated was 0.1 µg x 1/10 = **0.01 µg**

❑   Recall that each isolated colony started out from a single transformed cell. Use the number of colonies you counted (Table 4.3) to calculate the transformation efficiency for the plates containing LB + ampicillin + X-gal + IPTG for both types of transformed cells.

Transformation efficiency = _____ cells/µg of DNA for cells with plasmid without insert

Transformation efficiency = _____ cells/µg of DNA for cells with plasmid with insert

?   Which cells should you choose for the purpose of sequencing the inserted fragment?

Electrophoresis of Plasmids Purified from Colonies

To verify the presence of the DNA insert in the cloning vector and to estimate the size of the insert we need to purify the plasmid and subject it to the same restriction endonucleases used in the ligation experiment in lab one. The purification of plasmids and the restriction digest have been done for you by the lab staff. You will conduct an electrophoresis of an agarose gel containing samples from purified plasmid restricted by *Eco*RI and *Hin*dIII. As a comparison, purified plasmid not digested by restriction enzymes will be loaded in the same gel. This study will be done by groups of three to four students, with up to five groups per lab section. Pairs of groups will share an electrophoresis power supply and certain other equipment.

Preparing an Agarose Gel

Assembly for Gel Pouring (See Figure 4.10)

❑ Place the Horizon™ 11•14 gel electrophoresis apparatus on a flat surface away from the edge of the work bench. Open the safety interlock lid. Place the ultra violet transparent (UVT) tray in the electrophoresis tank centered on the white support platform. The red stripes on the platform make it easier to load sample into the wells.

❑ Slide the metal dams into the V-grooves adjacent to the UVT tray. These dams act as a heat sink, to solidify the agarose quickly and prevent leaking. The right angle surface of each dam must fit flush against one end of the tray. Simultaneously push down on each dam gently to seat the sealing surface of the dams against the tray.

❑ Place the bullseye level in the center of the tray and turn the adjustable feet under the tank until the apparatus is level.

❑ Note that the positive (red capped) electrode is on the right end of the tank. Unplug the power supply and ascertain that the red and black power cables are detached.

❑ Insert a comb into the comb alignment slots on the left end of the tray, nearest the black (–) electrode. Orient the comb so the teeth are directly over a well-visualization strip. The apparatus is now ready for gel pouring.

Gel Pouring

❑ Remove the 1.0 % agarose solution from the 60°C water bath. Immediately pour all of the liquid agarose (about 80 ml) into the center of the UVT tray to fill the tray to the proper depth. Note: alert your lab instructor if the liquid agarose leaks around the casting dams.

❑ Use a pipette tip to remove any bubbles that form, especially around the comb teeth or in the path that DNA will take during electrophoresis.

❑ Do not move or jar the electrophoresis tank for about 20 minutes while the agarose is solidifying.

Filling the Tank with Electrophoresis Buffer

❑   When the agarose has set, remove the dams. Do not remove the comb yet. Rinse the dams with distilled water and wipe dry.

❑   Pour sufficient Tris-Borate/EDTA electrophoresis buffer (TBE) into the tank to cover the entire gel to a depth of about 1 mm. This will require about 700 ml. Note: it is important to use the minimum amount of buffer to cover the gel. With excessive buffer in the tank, current flow will be mainly through the buffer instead of the gel and your DNA will move slowly.

❑   Gently remove the comb. <u>To avoid tearing the gel</u>, wiggle the comb to free the teeth from the gel as you lift up on one side of the comb, then the other side.

❑   Inspect the four wells that you created in the gel by removing the comb. If air bubbles are present in any of the wells, use a micropipette to pick out the bubbles.

❑   Be sure that the wells are completely submerged. The gel is now ready to load with samples.
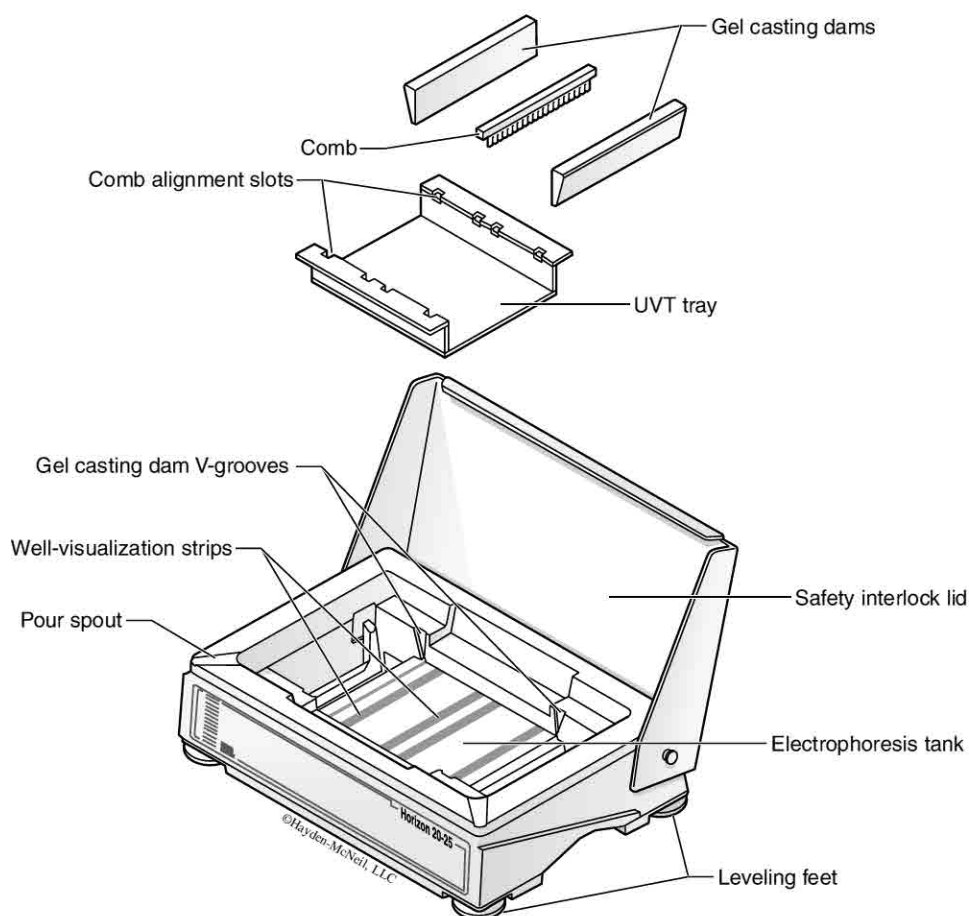
Figure 4.10. The Horizon™ 11•14 gel electrophoresis apparatus. See text for discussion.

Electrophoresis of DNA

Each group will receive three 1.5 ml microcentrifuge tubes containing the mixtures shown in Table 4.4.

Table 4.4. Tube contents and gel lane locations during electrophoresis.

TUBE	CONTENTS	DNA CONC.	LANE	BAND LOCATION (mm)						
				1	2	3	4	5	6	7
1	λ DNA , cut with *Hin*dIII	0.01 µg/µl	4*							
3	Uncut pUC8	0.01 µg/µl	3	—	—	—	—	—	—	—
2	Cut pUC8 + fragment	0.01 µg/µl	2							
1	λ DNA , cut with *Hin*dIII	0.01 µg/µl	1*							

* use the same micropipettor tip to load these lanes.

Contents of the tubes with restriction enzymes have already been digested. The fragments of DNA from the bacteriophage *lambda* (λ) in tube 1 will be used as size standards for estimating the fragment sizes of pUC8 and the genomic fragment.

Following the procedure described below, use a 20–200 µl micropipettor to load 25 µl of liquid from each tube into the designated well on your agarose gel. Note: with the wells on the left side of the gel, the well nearest to you is lane 1; the well farthest from you is lane 6.

❑ Set the micropipettor to 25 µl and fill it using the procedure you practiced earlier.

❑ With elbows resting on the bench top, hold the tip of the micropipettor in the TBE buffer directly over the well. The tip should be located just inside the well. Do not puncture the bottom of the well by inserting the tip too far into the well. Steadying the micropipettor with both hands may help.

❑ Slowly depress the plunger to release the liquid. The loading buffer contains a dense glycerol solution so the contents of the micropipettor will sink to the bottom of the well.

❑ Eject the tip from the micropipettor into an empty beaker and insert a clean one. Note: you can use the same tip for wells 1 and 4.

❑ Repeat this procedure until all four wells have been loaded. Now, close the safety interlock lid of the electrophoresis tank and connect the power cables to the tank electrodes, positive (red) to positive and negative (black) to negative. Now connect the cables to the appropriate receptacles on the power supply. You are ready to begin the electrophoresis run. Since two groups will be sharing an electrophoresis power unit, you may need to wait for the second group before proceeding to the next step.

❑ Plug in the power unit, select the "Hi" voltage setting, turn on the unit, and adjust the voltage to about 150 volts. When the unit is properly connected, small bubbles will start to rise from the black (–) electrode and the two blue bands of the loading dye will begin slowly moving toward the red (+) electrode.

❑ Allow electrophoresis to proceed for about 60 minutes.
❑ Turn off and unplug the power unit when the run is complete. Disconnect the power cables from the power unit first, and then from the electrophoresis tank.

❑   Note: the person doing the next step should wear disposable gloves. Open the lid and lift out the UVT tray and the gel. Slide the gel from the UTV tray into a shallow tray used for staining. Handle the gel with care; it will tear easily.

❑   Give the tray with your gel to the lab instructor. The lab instructor will stain the gel with a 1 µg/ml ethidium bromide solution. The gel will be photographed for you by the staff the next day. Caution: ethidium bromide is a mutagen and suspected carcinogen. Only your lab instructor should handle the gel during staining and photography.

❑   **CAUTION**: do not look directly at the UV light source when you view the gel on the transilluminator. UV light can damage your retinas. Be sure to close the cover of the transilluminator before turning on the light and looking at the gel. The cover blocks UV light but still allows you to observe the glowing DNA bands on the gel.

Once it has been verified that a particular colony contains the inserted fragment one may mark the colony and send the plate directly to a sequencing facility. The facility will need to know the cloning vector to determine the sequence upstream or downstream from the fragment insert to design a primer. The sequencing procedure is similar to that described in the section DNA Sequencing Using Sanger Method earlier.

Restriction Mapping of Plasmids

A plasmid **restriction map** shows the restriction sites for specific endonucleases accurately drawn to indicate the fragment sizes that will be generated. Data like those you collected today are used to construct restriction maps.

In your study of *Sordaria* in chapter 11, you obtained a **genetic map** showing the locations of the two spore color genes relative to their centromeres. Genetic maps are based on recombination data and correctly show the sequence of genes on chromosomes and their relative locations, but these maps do not portray physical distances. Recall that the units of a genetic map are % recombinant meiotic products to total meiotic products and are called centimorgans (cM). In contrast, a restriction map, based on DNA fragment analysis from a gel, shows the physical locations of specific restriction enzyme recognition sequences (restriction sites) on a DNA molecule, consequently a **physical map**. The units of restriction maps are in nucleotide base pairs (bp) or kilobase pairs (kb), where a kilobase is 1000 base pairs.

To obtain a restriction map you first digest a DNA molecule, such as a plasmid, or a portion of a DNA molecule with two or more restriction endonucleases, usually separately and in all possible combinations. Each reaction goes into a separate well. After electrophoresis, a standard curve is used to convert band migration distances into fragment sizes. The fragment sizes obtained in the various lanes of the gel constitute a set of observations from which a map can be inductively derived. The following steps are normally followed when restriction mapping a plasmid:

1.   The number of fragments in lanes from reactions with single restriction enzymes is the number of restriction sites for that enzyme present on the plasmid. One fragment results if a single restriction site exists, two fragments result if two restriction sites exist, etc. Note: if no restriction sites are present, then the bands obtained would be the same as those shown in uncut, control lanes (showing the relaxed and supercoiled conformations), which are also normally included on the gel.

2.   The total size of the plasmid is the sum of the estimated base pair sizes of the fragments in each lane. All lanes should yield about the same size estimate for the plasmid. (See note below.)

Note: 1 and 2 (above) will not apply if an enzyme produces multiple fragments of about the same size.

3. Draw a circle and arbitrarily place a mark representing the restriction site of an enzyme with a single site at the 12 o'clock position on the circle. (It helps to add additional marks that divide the circle into 10 equal segments).

4. Now look at a lane from a reaction with the enzyme you have placed on the circle and one other enzyme. Draw marks for the sites for the second enzyme on your circle scaled to reflect the fragment sizes in that lane relative to the mark for the first enzyme. (The fragment size divided by the total plasmid size times 10 is the number of units from the 12 o'clock position.) Write the fragment sizes next to all arcs created by the marks you have placed on the circle. The total of all fragment sizes should equal the estimated size of the plasmid.

5. Repeat this same process with the first enzyme and a third enzyme. To resolve where the marks for this third enzyme should be placed, you will need to look at the fragments obtained for it in combination with the second enzyme. Only one position will be consistent with all three enzymes.
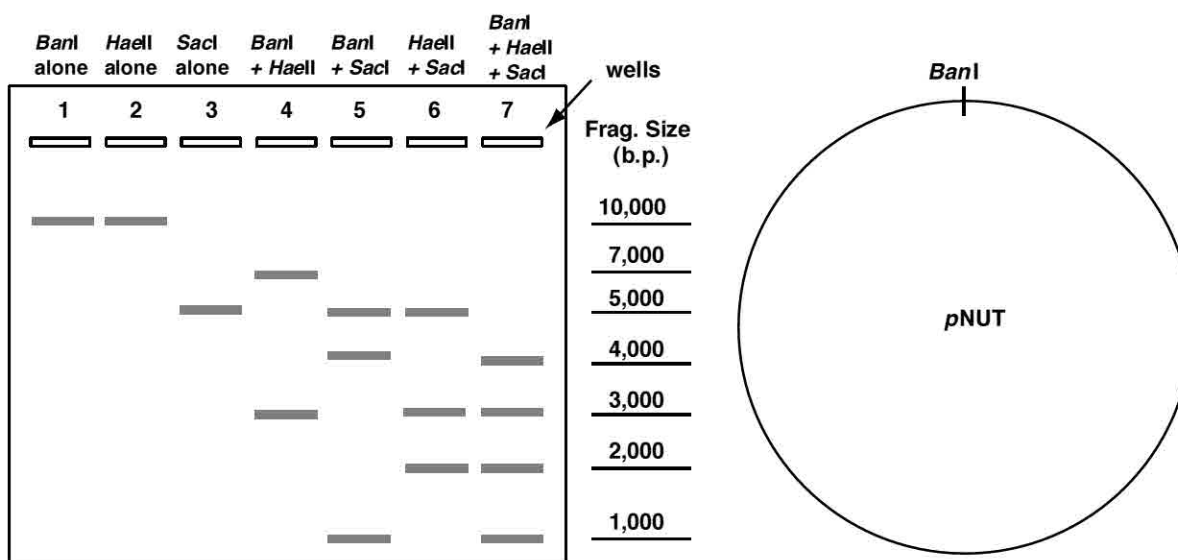
Figure 4.11. Electrophoresis gel resulting from a study to map the plasmid pNUT with the enzymes *Ban*I, *Hae*II, and *Sac*I.

6. With more than three enzymes or for enzymes with two or more restriction sites, the process becomes increasingly complex. Computer programs are useful for simplifying map construction.

As part of an in-class activity, you will produce a restriction map based on the gel shown in Figure 4.11. Additional problems associated with the worksheet will give you more experience with producing and interpreting restriction maps.

PROCEDURES - LAB THREE

TODAY SET-UP FOR ANGIOSPERM BIOASSAY

Read p. 169-170 and p. 175 of Chapter 8 in this manual to familiarize yourself with the procedures.

Using a Standard Curve to Estimate the Length of pUC8 Restriction Fragments

      You will receive a picture of the gel you ran during the last lab. In lanes 1 and 4 is the DNA from the bacteriophage λ cut by *Hin*dIII. DNA from phage λ is a linear, double-stranded molecule 48,502 base pairs in length. *Hin*dIII cuts this molecule into eight fragments, seven of which are observable with the electrophoresis system you used. The restriction sites and fragment sizes for λ DNA cut with *Hin*dIII are shown in Figure 4.12. Table 4.5 summarizes these data. The migration distances of these lambda fragments from bands observed in lanes 1 and 4 will be used to construct a standard curve that shows the relationship between fragment size in base pairs and migration distance in mm for the electrophoresis system you are using. This standard curve will then be used to estimate the sizes of pUC8 fragments from bands observed in lane 2.
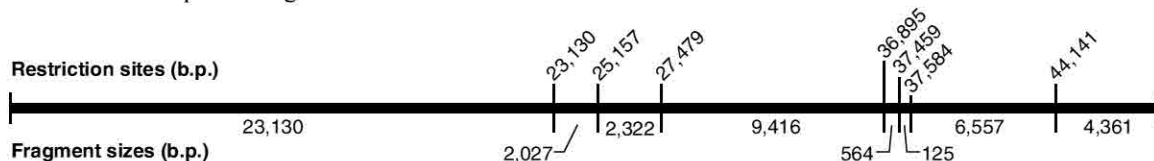
Figure 4.12. Restriction sites and fragment sizes in base pairs (b.p.) for λ DNA cut with *Hin*dIII.

❑   Use a small plastic ruler to make measurements directly on the photograph or photocopy of your gel. For each lane, determine the number of bands and measure to the nearest 0.5 mm the distance from the front edge of the well to the front edge of each band. Enter band location data for each lane in Table 4.4 from smallest to largest measurement.

❑   In Table 4.5, enter the average migration distances for the seven observable λ fragments from lanes 1 and 4 in Table 4.4.

      The best linear relationship between fragment size and migration distance is obtained by plotting both variables on logarithmic scales.

Table 4.5. Fragment sizes in base pairs (bp) for λ DNA restricted with *Hin*dIII and estimates of migration distances for these fragments.

Fragment Size (bp)	Migration Distance (mm)
23,130	
9,416	
6,557	
4,361	
2,322	
2,027	
564	
125	**Too small to be visible on this type gel.**
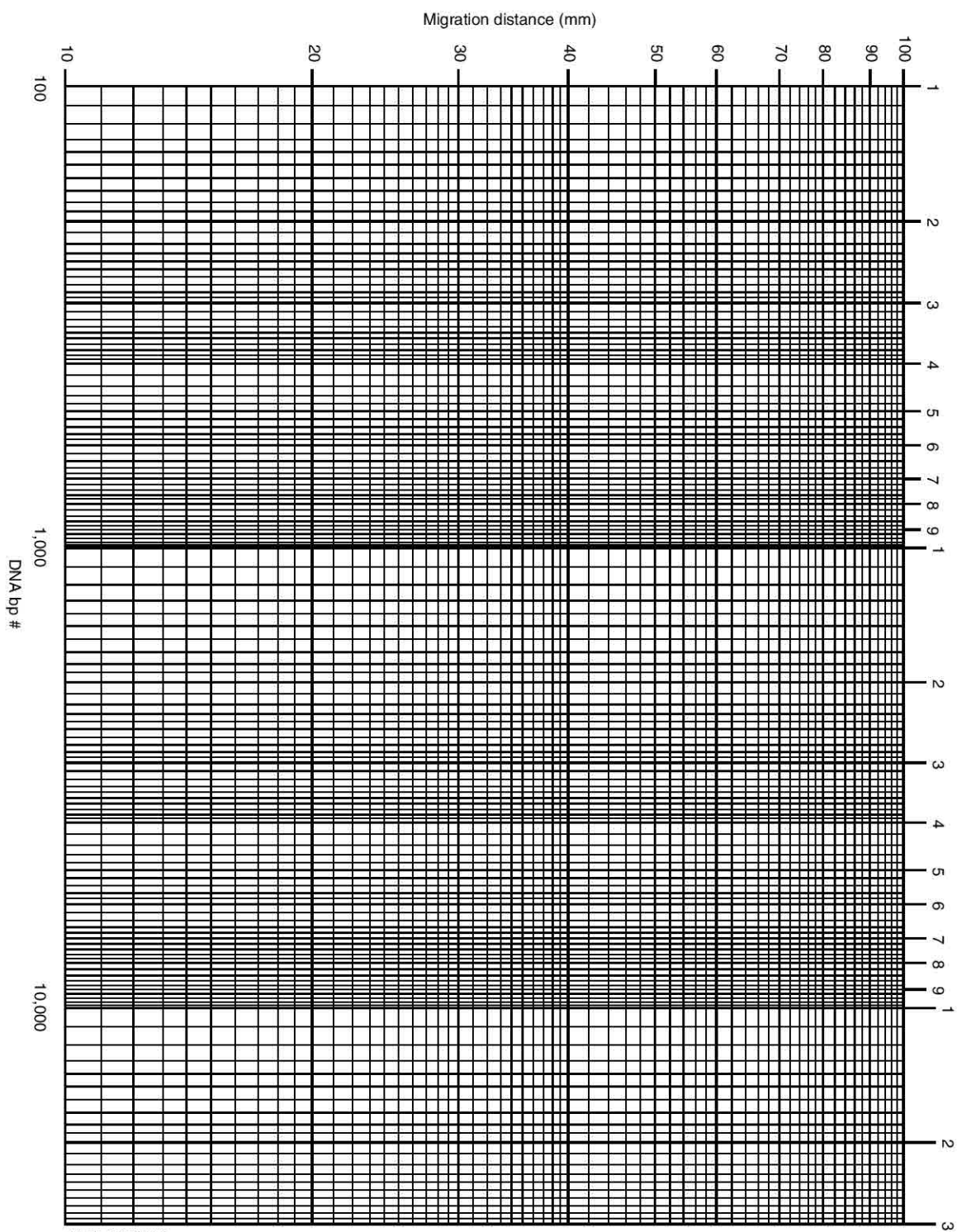
Figure 4.13. A standard curve showing the relationship between the log of the migration distance of λ DNA fragments in a 1% agarose electrophoresis gel and the log of fragment size in base pairs. The λ DNA was restricted with *Hind*III.

❑   Observe Figure 4.13, a log/log graph. Note that the y-axis (migration distance) is a single log cycle from 10 to 100. The x-axis (fragment size) includes two complete cycles and part of a third cycle, giving it a range from 100 to 30,000 base pairs. Major subdivisions of each cycle are given at the top of the graph. On the x-axis, line 3 in the first cycle is 300 and line 3 in the second cycle is 3000.

❑   Plot the data in Table 4.5 on the log/log graph in Figure 4.13. Place the edge of a ruler along the line that marks the size of a fragment and then plot the migration distance of that fragment.

❑   Ignore the data point from the largest fragment (23,130 bp) and draw a straight line that best fits the remaining data in Figure 4.13. There should be an approximate linear relationship between the log of fragment size and the log of fragment migration distances for the five fragments.

❑   Use the standard curve in Figure 4.13 to estimate the base pair size of the fragments in the bands you observed in lanes 2.

Sizes of fragments in lane 2: _____

❑   Estimate the amount of DNA in any one band visible in the gel picture. First, determine the total mass in µg of λ DNA by multiplying the concentration and volume loaded into the well. The amount of DNA of any one band is a fraction of this quantity based on its number of base pairs to the total λ DNA base pairs. Recall that the DNA concentration added to each well was 0.01 µg/µl, the volume added was 25 µl, and the total λ DNA is 48,502 base pairs.

$$\text{DNA mass} = \frac{\text{fragment bp (conc. DNA) (vol. DNA)}}{\text{total } \lambda \text{DNA bp}}$$

DNA mass of _____ bp fragment =

DNA's Conformation Affects Its Migration in a Gel.

The linear relationship between the log of DNA fragment size and log of fragment migration distances in an electrophoresis gel applies only to the type of linear DNA fragments produced by restriction endonucleases. Intact, circular plasmid DNA exists naturally in several forms, including conformations called **supercoiled circular** and **relaxed circular**. Supercoiled circular DNA is double stranded DNA that is twisted about itself like one can twist a loop of rope. Both DNA strands are intact in supercoiled DNA. If one of the strands of supercoiled DNA is cut, the tortional strain that keeps the molecule in a supercoil is released and the molecule uncoils into the relaxed circular form. The formation of relaxed circular DNA generally is caused by cutting one DNA strand during the plasmid isolation procedure. Because of its compact shape, the supercoiled form of a plasmid migrates faster through an electrophoresis gel than the relaxed circular form does.

Linear DNA is produced when a restriction endonuclease cuts both DNA strands of a plasmid at a single recognition site. Generally linear plasmid DNA migrates in a gel at a rate that is intermediate between that of its supercoiled and relaxed circular counterparts.

❑   In lanes 3, identify the bands representing the **relaxed circular, supercoiled circular, and linear** conformations discussed above. Are all conformations present?

Search for Nucleotide Sequence Similarity Using BLAST

You will conduct this exercise on your own at home or in a library. **Your work will be checked by the lab instructors at the beginning of the next lab.** Similar ecercises will also appear on the next quiz and practical exam. From your lab instructor you will receive a copy of an electropherogram with signal peaks of nucleotides shown in four colors . Choose the portion that shows clear signals. Use a word processing software such as Microsoft® Word to record about 400 bases of the sequence on your computer. Do not include spaces between nucleotides or hit the Return key.

❑   Record the sequence for about 400 bases on your computer.

❑   Go to http://www.ncbi.nlm.nih.gov/ and click BLAST at near the top of the page. You will be connected to a page with many choices of database search. Choose **<u>nucleotide blast</u>** under **Basic BLAST**. Copy and paste the sequence into the window under Enter Query Sequence. Choose "Others (nr etc.)" under Database, and "Somewhat similar sequences (blastn)" under Optimize for. Click the BLAST button.

A color chart listing the best sequence alignments will show up near the top of the results page. You may explore the best hit by mousing over the color bars. Alternatively you may scroll down the page and see the alignment list directly. The best sequence alignment match is usually at the top. Choose the best protein match (not a synthetic sequence, etc.).

?   Describe what gene this sequence is coding for and its source species.

_____

?   What is the Identity percentage between the queried and the matched sequences? _____%

A gap is indicated by a "-" in either the queried sequence or the hit sequence.

?   How many gaps are there in this alignment? _____

❑   Conduct another search by going back to the BLAST homepage. Choose <u>tblastx</u> for matches in translated nucleotide sequences. Copy and paste the sequence into the same window and click the BLAST button.

At the top section of the results is the reference citation of BLAST followed by the databases searched. A color chart listing the best sequence alignments follows. Below the color chart is the list of sequences with the highest level of similarity at the top.

?   Is the top hit the sequence coding for the same gene as that from the previous search?

❑   Record the two values used for assessing the alignment similarity between the queried and the sequence from the best match.

The Score of this alignment: _____. The E value of this alignment: _____

Under the *Sequences producing significant alignments* section is a list of sequences that align well with the sequence you input. In addition to the top hit, there may be genes from species other than the one with the best match.

❑   Are there any other good matches with genes from another species? List two of them here.

Gene: _____ Species: _____

Gene: _____ Species: _____

POST-LAB WEB ASSIGNMENT

To test your understanding of this laboratory, complete the associated web activities located in the lab section of the course web site. You will encounter questions similar to these on lab exams.

URL = http://biog-1101-1104.bio.cornell.edu/BioG1101_1104/tutorials/recomb_DNA.html

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BioG 1108   Name _____

                                            Lab Instructor _____

RESTRICTION MAPPING WORKSHEET[*]

1. A new plasmid (*p*New) is digested with the following combination of three restriction endonucleases:

*Pst*I	*Eco*RI	*Sal*I
*Eco*RI + *Sal*I	*Eco*RI + *Pst*I	*Pst*I + *Sal*I

Also shown are the base pair sizes of all fragments produced with the various reaction mixtures.

Use these data to produce a restriction map on the circle next to the gel. Show the restriction sites of the various enzymes and the base pair distances between all sites. For orientation, the *Pst*I site is already placed on the circle.

What is the total size of this plasmid in **kilobase pairs**? _____ kb
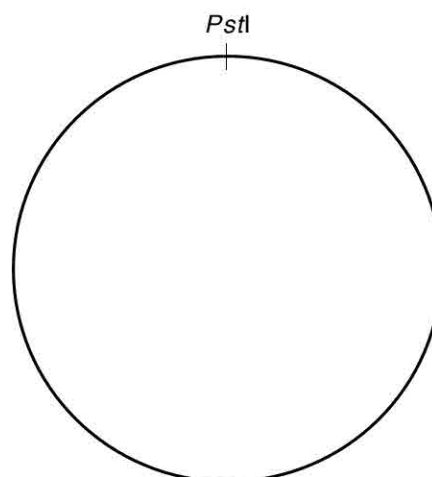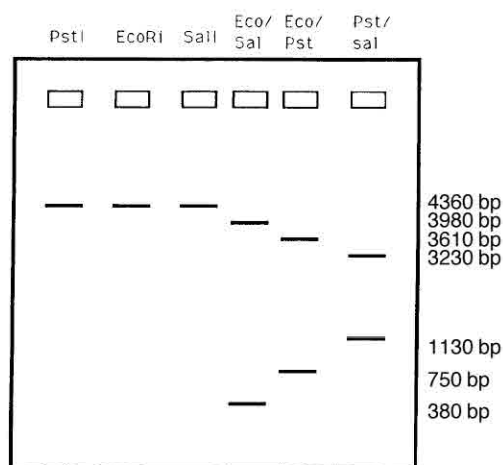
2. The following figure shows the restriction map for plasmid Y (total size = 8.0 kilobase pairs) with the endonucleases *Bam*HI and *Eco*RI. Use this map to complete the table.
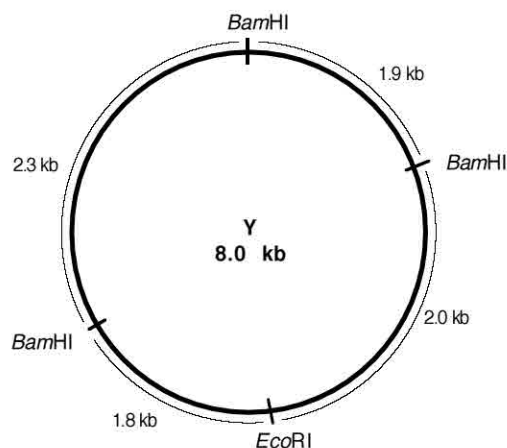
Digest Performed	Sizes of Fragments Obtained
*Eco*RI	
*Bam*HI	
*Eco*RI + *Bam*HI	

---

[*]See Restriction Mapping of Plasmids section for an approach to use in answering these questions.

3.   You digest a plasmid with restriction endonuclease, *Pvu*I, either alone or in combination with *Bam*HI and *Hin*dIII. The following gel results.

A.   What is the total size of the plasmid? _____

B.   On the circle to the right of the gel, draw a restriction map that is in agreement with these data showing the recognition sites of all enzymes. Show the base pair size of all fragments.

4.   A plasmid called *p*UC23 is digested with combinations of the enzymes *Eco*RI and *Bam*HI. Use the data shown in the following table to draw a restriction map for this plasmid. What is its total size?

Restriction Enzymes	Fragments Obtained (kb)
*Eco*RI	20
*Bam*HI	11, 6, 3
*Eco*RI + *Bam*HI	8, 6, 3
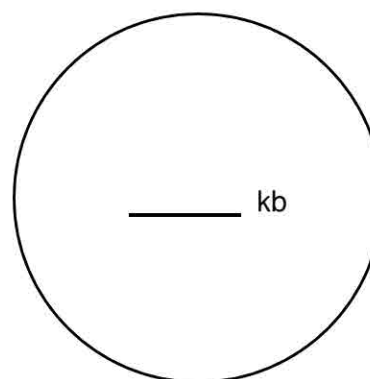
What would you predict about the fluorescence intensity of the band representing the 3 kb fragment in the lane containing the *Eco*RI + *Bam*HI digest? Explain your answer.

CHAPTER 5 – SEA URCHIN FERTILIZATION AND EARLY DEVELOPMENT

LABORATORY SYNOPSIS

In this laboratory you will become familiar with the pattern of development in a group of animals called the **deuterostomes**. Specifically, you will observe fertilization and early development in the sea urchin (*Arbacia punctulata*) and consider some of the underlying molecular mechanisms controlling these events.

LABORATORY OBJECTIVES

After completing this laboratory, you should&

1. have observed and be able to describe the external morphology of the sea urchin.

2. have observed and be able to describe the gametes of the sea urchin.

3. be able to describe the events of fertilization in the sea urchin, both those that you observed in lab and the underlying molecular mechanisms.

4. have observed and be able to describe the sequence of events that leads from a fertilized sea urchin egg to a pluteus larva, including early cell division, blastula, gastrula, and pluteus stages.

Reading assignment—COMPLETE THE FOLLOWING READINGS BEFORE COMING TO LAB.

Read this lab chapter.

Jon C Glase

Revised June 2010
Mark A. Sarvary

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

1. What is the effect of yolk distribution within the zygote on the observed pattern of development in animals?

2. What is the importance of the blastopore to the formation of the gastrula and in what way does the fate of the blastapore differ between deuterostomes and protostomes?

3. What are the differences in the genetic and cytoplasmic contribution of sperm and egg to the zygote?

4. What are the fast and slow blocks to polyspermy?

5. What is the importance of the pluteus larva to the life cycle of the sea urchin?

Background

Animal Developmental Pattern **(Figure 5.1). Fertilization** involves fusion of haploid sex cells (**gametes**—sperm and egg) to produce a diploid **zygote**. The zygote then undergoes a series of programmed mitotic divisions to produce the multicellular organism. In addition to cell division and cell expansion, development consists of differentiation (the generation of cellular specificity) and morphogenesis (the production of body form). In the animals, division of the zygote, called **cleavage**, gives rise to a hollow sphere of cells called a **blastula**. The individual cells of the blastula are called **blastomeres** and the cavity within the blastula is the **blastocoel**. Infolding of the blastula, called **gastrulation**, forms a tube-within-a-tube body plan called a **gastrula**. The opening to the newly formed gut tube is the **blastopore** and its inner cavity is the **archenteron**. The outer cell layer of the embryo is the **ectoderm**; the cell layer of the gut tube is **endoderm**, and **mesoderm** forms between endoderm and ectoderm.

The amount and distribution of lipid-rich nutrients called **yolk** dramatically determine the type of blastula and gastrula that develop in different animals. Yolk inhibits cell division and if the yolk is unequally distributed within an egg, cleavage takes place more readily in the yolk-free cytoplasm of the **animal pole** (as opposed to the **vegetal pole**). To understand the effects of yolk on development, review the patterns of early cleavage seen in the sea urchin, the frog, and the chicken discussed in your lecture text.

Two other developmental terms are worth differentiating. An **embryo** is a young animal or plant that is still contained within a protective structure, such as a seed coat or egg membrane (i.e. vitelline membrane or chorion). When an animal hatches from the egg and moves around freely, but is still morphologically different from the adult stage, it is called a **larva**.

There are similarities in several functions necessary for survival of the embryos of both the sea urchin and of amniotic animals. Both sea urchins and amniotes are Deuterostomes, and in both cases the zygote and early embryo is surrounded by an extracellualar matrix, referred to as either a **vitelline membrane**, fertilization membrane, or *zona pellucida*. This layer serves to protect the early embryo. In this way it functions in a manner similar to how the **chorion** acts to protect the embryo in those deuterostomes who are amniotes. Both the vitelline membrane and the chorion offer protection, but the vitelline membrane is an extracellular matrix, while the chorion is made of sheets of cells derived from the zygote but which are not part of the embryo. In addition to the chorion, another extraembryonic membrane in amniotes also serves an analogous function to an item in the sea urchin early embryo. The **yolk sac** of amniotes is an extraembryonic membrane which serves to help provide nourishment to the developing embryo from either an extraembryonic mass of yolk (think of a chicken egg) or from a placenta (as in humans). This is analogous to the yolk in early embryo or such animals at sea urchins or frogs which ends up mainly in a structure called a yolk plug during gastrulation, in that it also supplies nourishment to the growing embryo. Thus a study of the sea urchin's embryonic development can illustrate some functional ways it is similar to that of our own development.
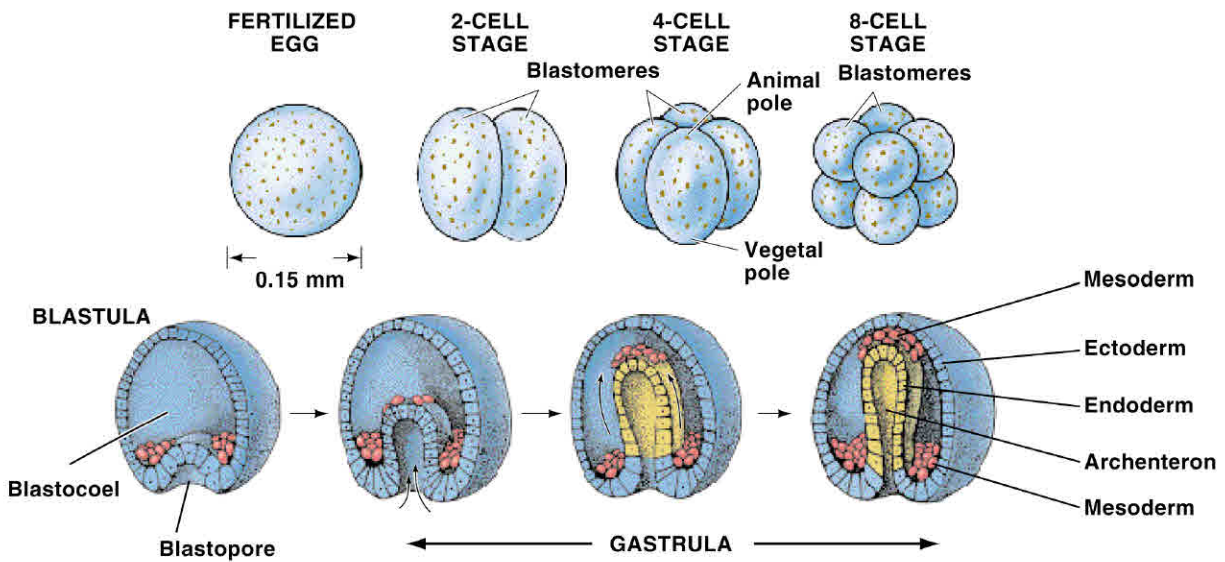
Figure 5.1. Animal development as shown by the sea urchin.

The Deuterostomes. The deuterostomes and protostomes represent the two major lineages of the animal phylogenetic tree (see Figure 32.10 on page 662 of *Biology* (Campbell and Reece 2008). Protostomes include the worms (flat, round, and segmented), the mollusks, and the arthropods. The deuterostomes include the echinoderms and the chordates. Most of the differences between protostomes and deuterostomes are developmental, including the type of cleavage, how mesoderm is formed, and the origin of the body cavity (**coelom**). Another major difference relates to the developmental fate of the blastopore (opening into the archenteron). In the protostomes, the blastopore becomes the mouth and the anus forms later; protostome means, *first-mouth.* In the deuterostomes, the blastopore becomes the anus, and the mouth forms later; deuterostome means, *second-mouth.* In addition to sea urchins, major types of echinoderms include sea stars, brittle stars, crinoids, and sea cucumbers. Although there are several minor groups of invertebrate chordates, the vertebrates represent the majority of the chordates. Today you will have an opportunity to study the gametes, embryos, and larvae of a representative echinoderm, the sea urchin.

Fertilization and Early Development in Sea Urchins

Sea Urchin Natural History. Sea urchins are a class in the phylum Echinodermata. Like all echinoderms, sea urchins are entirely marine, living as adults on the ocean floor from the intertidal, subtidal, to deep ocean zones. All echinoderms have radial symmetry as adults, but, as you will see, as embryos are bilaterally symmetrical. There are about 700 species of sea urchins extant today. In the laboratory you will be studying *Arbacia punctulata*, a species that breeds from October to April in the Gulf of Mexico.

Most sea urchin species are herbivores and in areas where their own predators are reduced, urchins may overgraze and destroy their habitat. Pacific Coast species prefer various species of kelp, but will consume a variety of algae. Fish (such as the parrot fish) sea stars, gulls, and sea otters help to control urchin population density. Sea urchin gonads are regularly eaten by humans. The annual harvest of sea urchins in the state of California is about 20 million tons, shipped mostly to markets in Japan. Sea urchins are popular laboratory animals because they are easy to harvest, produce many gametes, and fertilization is external, taking place in seawater. The eggs are about 100 μm in diameter and are barely macroscopic. During the breeding season, gonads may represent 60% of an urchin's body weight. Much of what is known about the molecular events of fertilization and early development was first established through study of sea urchins and their relatives the sea stars.

All sea urchin species are dioecious (individuals are either male or female), but the sexes cannot be distinguished externally. Urchins tend to live in colonies and at certain times of the year, specific for each species, gametes are released by the colony. In its lifetime, a female may produce one billion eggs and a male, 10 billion sperm. Eggs secrete chemicals unique for each species that are attractive to sperm and orient their swimming. After fertilization and about five days of development, an actively-swimming **pluteus larva** appears. Swimming is by cilia and the pluteus larva feeds and disperses for about two to three months before it settles to the bottom, undergoes metamorphosis, and becomes a radially symmetric adult. In the laboratory, spawning is induced by injecting 0.5 M KCl into the body cavity to cause contraction of the gonads and release of gametes.

Sea Urchin Morphology. Adult echinoderms have a body plan with parts arranged around a central axis. The upper or **aboral** surface has openings for the gonads (ovaries or testes), the mouth is located on the lower or **oral** surface. An inner skeleton of calcareous plates is covered by a thin layer of skin and some muscles. The skeleton of a sea urchin is called its **test**.

❑   Examine a sea urchin test. Note that it consists of a number of plates that have been fused together. The sea urchin's numerous **spines** are attached to rows of small elevated cones on each plate. Muscles allow the spines to be moved individually. They serve both a protective and locomotory function. Hold the test up to the light and note the rows of small holes along the borders of the plates, oriented in an oral-aboral direction. **Tube feet**, the effector organs of the water vascular system, emerge through these holes. The water vascular system is a series of seawater-filled, hydraulic canals used to extend and retract the tube feet. A tube foot is extended and anchored with its suction cup end, and when it retracts a tiny pull is exerted on the sea urchin. The coordinated action of all of the tube feet (and the spines) allows the urchin to move slowly along the sea floor in search of food or shelter.

❑   Examine a live sea urchin and identify the spines and tube feet. Note: the tube feet are difficult to see unless the animal is left undisturbed in seawater. Turn the animal upside down to view the oral surface. Note the mouth and the five protrusable teeth. The teeth are used to scrape and chew. They are part of an internal set of calcareous structures making up a jaw apparatus called Aristotle's lantern. Surrounding the mouth is a soft membrane called the **peristome** that has its own oral tube feet. The peristome has a chemosensory function.

Obtaining Gametes. So we use as few animals as possible, collection of gametes will be done by the prep staff. A single male and female can provide enough sperm and eggs for all lab sections meeting at one time.

❑   View a short videotape on gamete collection.

Each sea urchin is injected with 0.5 M potassium chloride through the peristome membrane around the mouth into the animal's body cavity. Animals are then gently shaken for a few seconds to mix the KCl and induce contraction of the gonads. Gametes are expelled through genital pores on the upper, aboral surface. Males produce creamy white sperm; females produce pale yellow to orange eggs, depending on the species. Once the sex is determined, males are inverted over a small petri dish and the sperm are collected in a "dry" condition. Females are inverted over a small beaker filled with seawater so that the eggs are shed into the seawater.

Sperm will be delivered to the lab in an undiluted condition. The sperm can be refrigerated (4 °C) in this condition for several days. Sperm become activated when placed into seawater, but are only viable for several minutes after activation. Your lab instructor will dilute sperm for the class when you are ready to use them. Eggs will be diluted with seawater to produce an appropriate density for you by the prep staff. However, because the eggs quickly settle, be sure to **mix** the egg stock suspension before you remove a sample.

Sea Urchin Gametes. Sea urchin gametes are about the size of human egg and sperm and their morphology is similar. The sea urchin sperm cell, minus its flagellum or tail, is 5 *μ*m in length. The sea urchin egg is 100–150 *μ*m in diameter. You will observe both.

Sea urchin sperm (Figure 5.2).

Sperm consist of three regions. The **head** is tipped with an acrosome containing enzymes that enable the sperm to digest its way through the egg's jelly coat. The acrosome also contains some globular actin that polymerizes to form an **acrosomal process** that penetrates the jelly and makes contact with the egg's **vitelline membrane**. Receptors on the membrane bind to species-specific **bindin** molecules on the outside of the sperm's acrosomal process. The posterior portion of the sperm head contains the haploid nucleus and a single centrosome (an organelle that organizes microtubules during cell division). The sperm's **midpiece** contains mitochondria that provide the energy for swimming. And the **tail** region is a single flagellum. Sperm swim by rotating their flagellum in a spiral, three-dimensional motion.

During fertilization, the sperm nucleus and single centrosome enter the egg, but the midpiece and the tail are discarded.
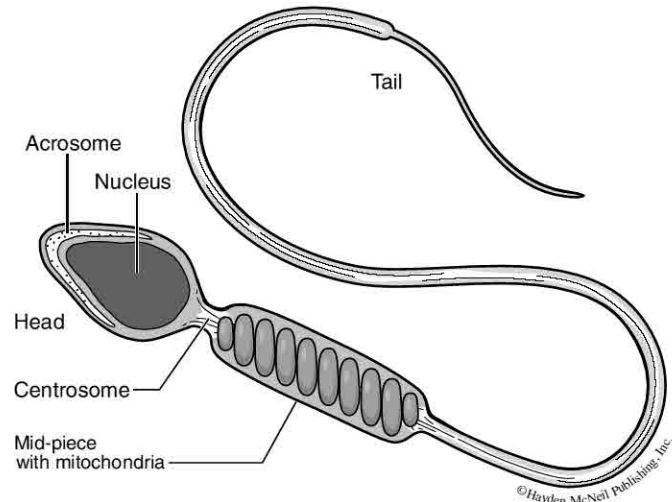
Figure 5.2. Human sperm cell.

Observation of eggs.

The egg consists of an outer vitelline envelope, covered with a thick jelly layer, and an inner cell (plasma) membrane. In the unfertilized egg, the vitelline envelope is tightly bound to the plasma membrane. Recall that during the meiotic division of the female germ cell (oogonium), one large haploid cell with most of the cytoplasm is formed (the "egg"), plus three **polar bodies** (small cells each with a haploid nucleus and very little cytoplasm). This allows most of the energy, raw materials, and RNA to end up in the egg.

❑   Place several drops of <u>well-mixed</u> egg suspension into the depression of a depression slide and carefully lower a coverslip on to the preparation. View at 100´ magnification. How big is the egg? Recall that a typical compound microscope with 100´ magnification has a viewing area with a diameter of about 1.8 mm (~1800 *µ*m). Make notes on what you can observe.

❑   Carefully remove the coverslip and add a drop of Sumi ink to the egg suspension. You may need to add a drop or two of additional egg suspension to the depression, but do not get any ink into the stock egg container—it will kill eggs!

❑   Mix the ink, replace the coverslip, and observe again at 100´ magnification. What can you see that you could not see before? The halo around the egg shows the extent of the egg's jelly layer.

❑   Dispose of the coverslip in the "Used Coverslip" container and <u>thoroughly</u> wash, rinse, and dry the microscope slide.

Fertilization. Review the information about the events of fertilization on pages 1022–1023 in your lecture text *Biology* (Campbell and Reece 2008). This paragraph summarizes the information presented there. Upon contact by the sperm with the egg, the acrosome membrane breaks down and the digestive enzymes are released. Acrosomal enzymes digest the jelly coat covering the egg. The **acrosomal process** is formed by polymerization of the globular actin. When the acrosomal process contacts the vitelline envelope of the egg, its bindin proteins complex with receptors on the envelope. Binding of the sperm acrosomal process to the sperm receptors that extend through the vitelline envelope causes an outpocketing of the egg's plasma membrane to form a **fertilization cone**, which will become the entry point for the sperm into the egg. At this time, the first stage of fertilization **plasmogamy**, has been completed. Next the egg also undergoes a rapid electrical depolarization due to an influx of sodium ions into the egg. Starting at the point of sperm entry, calcium ions that had been sequestered within the endoplasmic reticulum are now released into the cytoplasm. This wave of release of Ca^{++} spreads across the egg and causes **exocytosis** of cortical vesicles into the space between the plasma membrane and the vitelline envelope. The enzymes released from the vesicles break the bonds holding the envelope to the plasma membrane allowing an influx of water into the space. This raises the vitelline envelope, which becomes a distinct membrane called the **fertilization membrane**. The **fast block** to polyspermy is the depolarization of the egg cell membrane. The **slow block** is the raising of the fertilization membrane. Both prevent entry of more than one sperm into the egg. See Figure 5.3 for a summary of these events.

Sperm contacts egg (t = 0 sec)

Acrosomal reaction (t = 1 sec)

Acrosome membrane breaks

Enzymes released

Jelly layer digested

Actin filaments polymerize

Acrosomal process forms

Process binds to sperm-binding receptors on vitelline envelope

Outpocketing of plasma membrane forms "fertilization cone"

"Fast block" to polyspermy (t = 2 sec)

"Slow block" to polyspermy (t = 30 sec)

Sperm and egg plasma membranes fuse

Na^+ (or Ca^{++}) channels open in egg plasma membrane

Depolarization of plasma membrane

Sperm nucleus enters egg

Karyogamy (t = 20 min)

Metabolic activation of egg

Ca^{++} released from ER

Exocytosis of cortical vesicles

Enzymes released into perivitelline space

Vitelline and plasma membranes separate

"Fertilization" membrane forms

Sperm-binding receptors released

First mitotic division of zygote (t = 90 min)

Further development

Figure 5.3. A summary of the major events and timing of fertilization and early development in the sea urchin.

Observation of Fertilization

❑   Place several drops of <u>well-mixed</u> egg suspension into the depression of a depression slide. Without a coverslip in place, focus on some eggs and adjust the light intensity appropriately.

❑   When all is ready, use an eyedropper to add one drop of diluted sperm (from a batch freshly prepared for you by the instructor) to the egg suspension in the depression and mix. Quickly add a coverslip to the preparation and refocus on the eggs. Low light intensity will make the sperm more visible. Dilution activates the sperm, they become motile, and capable of interacting with an egg. This is called **capacitation**.

❑   You should see sperm swarming around each egg. After a few minutes you should also see a <u>fertilization membrane</u> develop around each egg. The development of a fertilization membrane is indicated by the appearance of a distinct layer around the egg and is due to the separation of the vitelline layer from the plasma membrane.

❑   Using transparencies of figures from a textbook, your lab instructor will review the events of fertilization with you. You will also have an opportunity to look at some video sequences made using special microscopy techniques that allow visualization of some of these events. These QuickTime® movies document research done on fertilization by Dr. Mark Terasaki at the University of Connecticut Health Center (1998) and are available from the web site at http://terasaki.uchc.edu. A link to this site is provided from the lab pages of the course web site.

Early Development. Following fertilization, each zygote undergoes many cleavages to form a multicellular embryo. During the first several cleavages there is no cytoplasmic growth of the embryo; the initial volume of the egg stays about the same and is simply divided up into more and more cells. In sea urchins, the first cleavage of the zygote occurs 60-90 minutes after fertilization. The second cleavage occurs 20-40 minutes later. Your lab instructor fertilized some sea urchin eggs at the beginning of the lab session. You will look at these embryos to see early embryonic stages. You will also have access to embryos resulting from eggs fertilized earlier in the week by other lab sections.

❑   Add several zygotes from the batch fertilized by your lab instructor to a depression on a depression slide and add a coverslip.

?   Has cleavage occurred? What is the plane of the first cleavage?

?   Has a second cleavage occurred? What is its orientation relative to the first?

❑   Your class will now work cooperatively to look at other embryos that are available to you. Each of you will take responsibility for setting up a microscopic preparation of 1–2 embryos of known age using the method described above. Label your microscope as to the age of the embryos. Be sure to study each microscopic setup.

❑   Observe and try to estimate the time since fertilization when:

"   a blastula first appears (see Figure 5.4, left).

"   movement of the blastula within the fertilization membrane is evident.

"   the blastula has "hatched" from the fertilization membrane.

"   a pluteus larva first appears (see Figure 5.4, right).
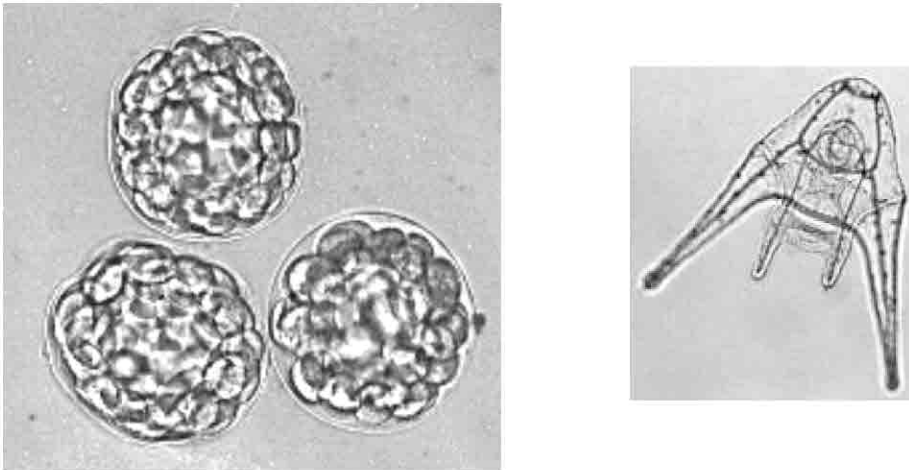
Figure 5.4. Left, early blastula (24 hours) still within the fertilization membrane; right, pluteus larva at about 5 days. Both pictures from web materials on sea urchin embryology developed at Stanford University—© 1998 Leland Stanford Junior University (see http://www.stanford.edu/group/Urchin).

FRESHWATER ECOLOGY LAB SET-UP

IN THE SECOND HALF OF THIS LAB YOU WILL SET UP THE FRESHWATER ECOLOGY EXPERIMENT (CHAPTER 10). PLEASE READ THE METHODOLOGY (DAY 1) OF CHAPTER 10 TO MAKE SURE THAT YOU ARE FAMILIAR WITH THE PROCEDURE.

PLEASE REVISIT THE FRESHWATER ECOLOGY INTRODUCTION THAT WAS GIVEN IN AN EARLIER LAB.

SETTING-UP THE FRESHWATER ECOLOGY LAB WILL TAKE SIGNIFICANT AMOUNT OF TIME. WITHOUT BEING FAMILIAR WITH THE METHODOLOGY IN ADVANCE, YOU MAY NOT BE ABLE TO COMPLETE THE LABORATORY REQUIREMENTS.

REFERENCES AND SUGGESTED READINGS

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CHAPTER 6 – DIVERSITY IN PLANT MORPHOLOGY AND LIFE CYCLES

LABORATORY SYNOPSIS

This laboratory addresses the evolution of diversity in morphology and life cycles of nonvascular and vascular plants. Specimens from selected phyla of plants will be displayed. You will study the life cycles of mosses and ferns and the morphology of various stages in those life cycles. You also will study the morphology of reproductive structures of a coniferous gymnosperm and of angiosperms within the context of their life cycles. Major evolutionary changes in the life cycles of plants will be emphasized and summarized in a phylogenetic tree.

Certain locations on the Cornell campus where you may observe plant diversity are given at the end of this chapter.

LABORATORY OBJECTIVES

1. Understand the difference between haploid and diploid cells, and know where in the sexual life cycle of plants these cell types are produced.

2. Understand the functions of the gametophyte and sporophyte stages in the plant life cycle, and know which of these stages is dominant in the life cycles of nonvascular and vascular plants.

3. Know the major differences between the nonvascular and the vascular plants.

4. Know the names of the phyla of plants presented in this laboratory and be able to place a plant in the appropriate phylum.

5. Know the function of each of the following structures: spore, sporangium, sporophyll, antheridium, archegonium, megaspore and microspore; whether the structure is part of the sporophyte or gametophyte generation; and the location of the structure in plants of the different phyla.

6. Understand the fundamental differences between homosporous and heterosporous plants, and the differences between megagametophytes and microgametophytes in the life cycle of a heterosporous plant.

7. Be able to recognize the sporophyte and gametophyte stages of a moss and a fern.

8   Know where the sporangia are located on/in the sporophyte of representatives from each plant phylum.

9. Know the main characteristics of seed plants that adapt them better than other vascular plants to an arid terrestrial environment.

10. Know what a seed and a cone is, and the origins of its components.

11. Know what a pollen grain is structurally and functionally.

12. Know what a flower is with respect to botanical structures.

13. Know the major parts of a complete flower and where meiosis occurs within the flower.

14. Understand the process of double fertilization in angiosperms, and how this process makes the angiosperm seed different from the gymnosperm seed.

15.   Know what a fruit is in terms of floral structures.

16. Be able to determine if an angiosperm is a monocot or a eudicot from its leaf venation pattern and/or flower structure.

17. Know the main features of the angiosperm life cycle that make it different from the gymnosperm life cycle.

18. For each phylum of plants considered be able to: Describe how it meets the need to disperse members of its species. Point out adaptive features that assist members of that groups to resist dry conditions. Relate the gamete-producing structures to the tendency for there to be inbreeding or outbreeding in each group of plants. Note how the set of characteristics in each phylum relates to the typical habitat in which it is found.

19. Understand the difference between fertilization and pollination.

NOTE: The Bioreview Sheets of life cycles used in this laboratory have many terms not used in the laboratory text. You should know only the terminology used in the laboratory text and specified in the preceding learning objectives.

READING ASSIGNMENTS (should be done before lab period)

In Campbell et al., *Biology*, 8th ed. (2008) read the overview of plant diversity.

Read this chapter.

                                                              Paul R. Ecklund

                                                              Revised June 2010
                                                              Scott Meissner and Mark A, Sarvary

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

1.   What does the term "alternation of generations" mean?

2.   Spores and gametes are single haploid cells. What functions do they perform in the plant life cycle?

3.   In the sexual life cycle of plants:

   A.   1.   What single cell gives rise to the gametophyte generation?

         2.   In what structure is this cell formed?

         3.   This cell is the product of which type of cell division, mitosis or meiosis?

B. 1. What single cell gives rise to the sporophyte generation?

    2. Is this cell haploid or diploid?

    3. In what plant structure is this cell located?

4. Describe the differences between a homosporous plant and a heterosporous plant:

    A. In the sporophyte generation

    B. In the gametophyte generation

INTRODUCTION

Overview and Objectives

In this cursory study of plant diversity you will examine specimens of representatives of the four plant phyla that are most frequently encountered in the Ithaca area: Bryophyta (mosses), Pterophyta (ferns), Coniferophyta (conifers) and Anthophyta (angiosperms/flowering plants). These four phyla, in turn, represent the four main groups of plants: bryophytes (nonvascular plants), pteridophytes (seedless vascular plants), gymnosperms (vascular plants that produce naked seeds) and angiosperms (vascular plants that produce flowers and enclosed seeds). The plant kingdom is a monophyletic group, which means it is derived from a common ancestor. Each group within the plant kingdom is, in turn, a monophyletic group. As you do this study, consider the differences observed in the life cycles of the four phyla as variations of a common ancestral life cycle.

After doing this study you should be able to identify the phylum of each of the specimens, identify certain structures on the specimens and know the role(s) of these structures in the life cycle of the plant. The four phyla presented represent the results of four major episodes in the evolution of plants as terrestrial organisms. Those episodes are summarized in a phylogenetic tree (Figure 6.3).

Plant Life Cycles

The sexual life cycle of all plants consists of an alternation of a <u>multicellular</u> haploid generation with a <u>multicellular</u> diploid generation. The haploid generation is called a **gametophyte** (gamete producing plant), which produces sperm cells in a structure called an **antheridium** and/or produces eggs in archegonia (one egg per **archegonium**). The diploid generation is called a **sporophyte** (spore producing plant), which produces spores in a **sporangium**. Within the sporangium, diploid **spore mother cells** undergo **meiosis** to form haploid spores. Each **spore** may develop into a gametophyte by mitotic cell divisions. A **zygote** (diploid cell) resulting from **syngamy** (fusion of egg and sperm) develops into a sporophyte by mitotic cell divisions. A generalized life cycle of a homosporous plant is shown in Figure 6.1. <u>Homo</u>sporous (producing <u>same</u> spores) plants produce only one type of spore which develops into a hermaphroditic (bisexual) gametophyte bearing both antheridia and archegonia. Most ferns are homosporous.
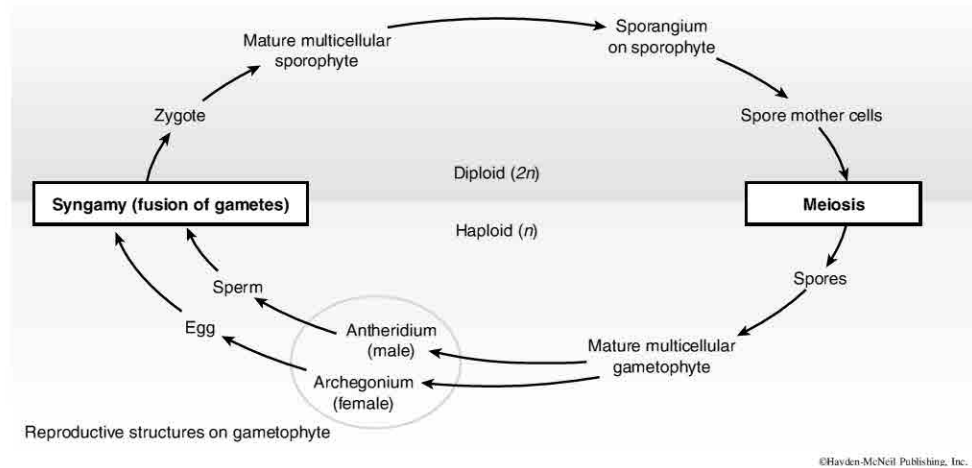
Figure 6.1. Generalized life cycle of a homosporous plant.

Figure 6.2. Shows the generalized life cycle of a seed plant which is <u>hetero</u>sporous (producing <u>different</u> spores) plant. Such plants produce two types of sporangia on the sporophyte and two types of haploid spores. Each spore gives rise to a <u>unisexual</u> gametophyte. **Microspores** develop into **microgametophytes** (male gametophytes), which produce sperm cells. **Megaspores** form **megagametophytes** (female gametophytes) with only archegonia containing eggs. All seed-producing plants and some ferns are heterosporous.
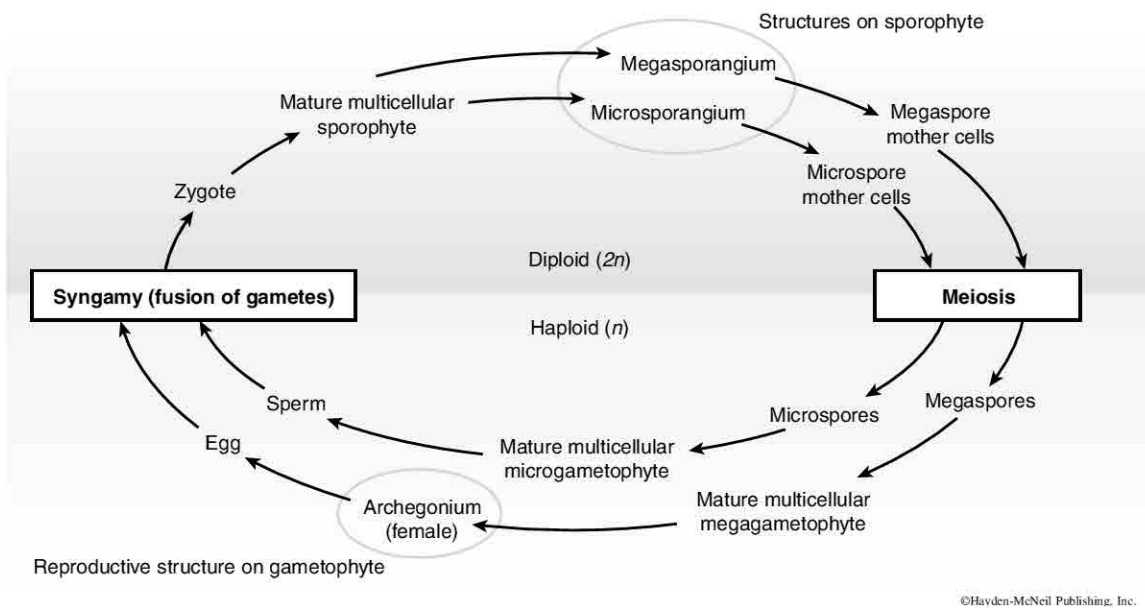
Figure 6.2. Generalized life cycle of a seed plant (a heterosporous plant). In both gymnosperms and angiosperms the microgametophyte is so reduced that it cannot produce an antheridium, though it still produces sperm. The megagametophyte in angiosperms also is reduced to the extent that an archegonium is lacking.

Origin of Plants

The many shared characteristics of plants and green algae have caused biologists to conclude that plants evolved from a type of green algae. This evolutionary relationship between green algae and plants was briefly discussed in the DIVERSITY OF PHOTOSYNTHETIC PIGMENTS laboratory in BioG 1107. Campbell and Reece (2008) describe several **shared primitive characters** which strongly suggest that plants and the charophycean group of the green algae had a common ancestor. Characters that distinguish plants from charophycean algae are called derived characters. An example of a shared primitive character in plants and the charophycean algae is the presence of multicellular archegonia on the multicellular haploid organism; therefore, we assume that their common ancestor had this character. Charophycean algae do not produce a multicellular diploid generation as plants do, so we infer that an **alternation of generations** did not occur in their common ancestor, and is a derived characteristic which evolved independently in plants. Another derived character of plants is the "embryo" stage of the sporophyte that remains attached to the parent plant and receives protection and nourishment from it.

Plant Groups Represented

Members of Bryophyta are called **nonvascular plants** because they have no vascular system or a very poorly developed vascular system. Ferns, conifers and flowering plants have well developed vascular systems composed of both xylem and phloem, and are called **vascular plants**. Ferns do not make seeds, and all terrestrial ferns are homosporous. Two groups of aquatic ferns are heterosporous. Conifers and angiosperms are seed plants and are heterosporous, a favorable condition for seed production. Coniferophyta and three other phyla of seed plants are collectively called gymnosperms because their members have "naked" (exposed) mature seeds on the seed producing structure. The mature seeds of flowering plants (angiosperms) are contained in the flower's ovary, which becomes a fruit. The seeds are not exposed until the fruit is ruptured, digested or otherwise decomposed.

PHYLOGENETIC TREE FOR THE PLANT GROUPS STUDIED

Figure 6.3 is a phylogenetic tree (or cladogram) to show the evolution of the four plant groups treated in this laboratory. The tree also includes an outgroup, the charophycean group of Chlorophyta (green algae), which is closely related to the four plant groups, but not as closely related as the plant groups are to each other. Characters shared by the charophycean green algae and the plants are called **shared primitive characters** presumably because they were present in a common ancestor. Each fork in the tree is determined by the presence of *shared derived characters*, which are common to all the species on one branch of a fork, but not shared by the species on the other branch. Presumably all the species with a distinguishing set of *shared derived characters* have a common ancestor. The *shared derived characters*, which distinguish plants from the charyophcean, Chlorophyta are given at position 1 on the cladogram. The shared derived characters distinguishing the vascular plants from bryophytes are given in position 2. The conifers are different from the conifers by having the shared derived characters listed in position 3. The flowering plants are different from the conifers by having the shared derived characters listed in position 4.
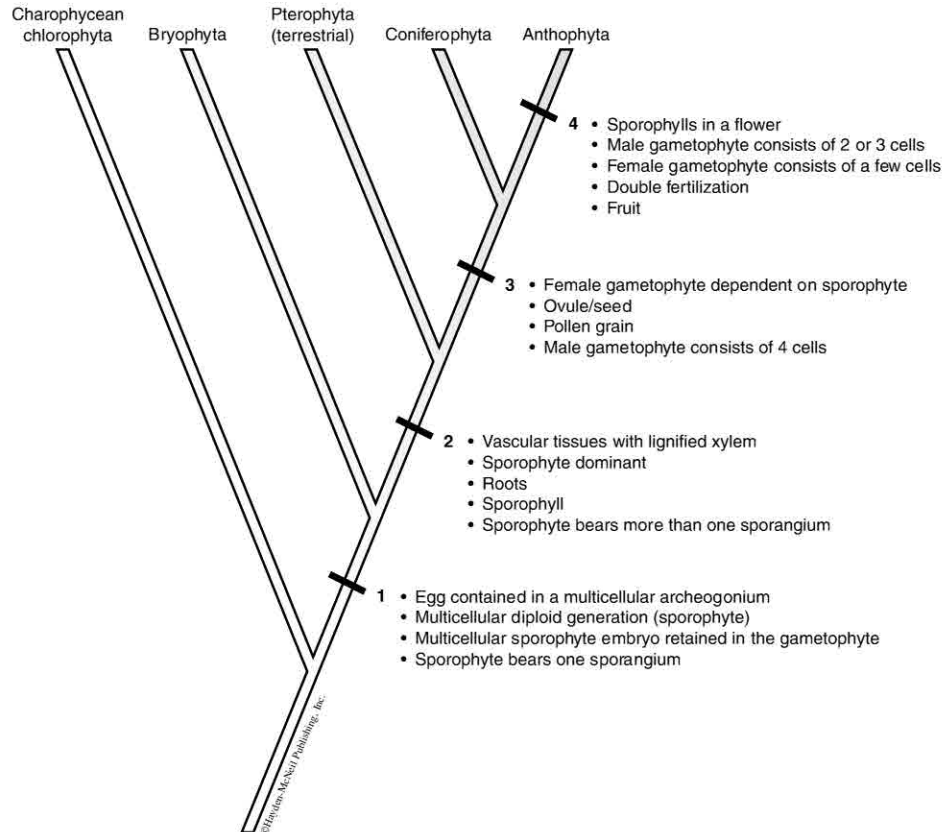
Figure 6.3. Phylogenetic tree for the plant groups studied.

The flowering plants are the most recently evolved phylum of plants. Since their appearance on earth, estimated to be around 120-130 million years ago, they have become the most successful and abundant of all plant groups. The number of existing species in Anthophyta is 350 times greater than the number of species in the four existing gymnosperm phyla. Until the late 1990s the flowering plants were classified into two major groups – monocots and dicots – based on the number of embryonic leaves, called **cotyledons**, present on the embryo in the seed. Monocot embryos possess one cotyledon, while dicot embryos have two cotyledons. Recent application of molecular systematics and cladistic analysis on the angiosperms reveals that they include at least four monophyletic groups (clades). The monocots are still regarded as a clade, but the former "dicots" have been separated into three clades. The majority of former "dicots" that are familiar to you are in the clade called eudicots. In addition to differences in cotyledon numbers, other, more enduring differences generally distinguish monocots from eudicots. The most conspicuous differences are the following:

Monocots: Leaves with parallel venation (i.e., vascular bundles run side-by-side along the length of the leaf).

Flower structures (e.g., sepals, petals, stamens) generally occur in threes or multiples of threes.

Eudicots: Leaves with net venation (i.e., vascular bundles branch and subbranch to produce a network of veins running in many directions).

Flower structures occur in twos, threes, fours or fives or multiples of those numbers.

Using Bioreview Sheets and prepared displays you will study the general morphology of both sporophyte and gametophyte stages of a moss and a fern. Through observation and dissection you will study the morphology of a flower and consider the flower within the context of the angiosperm life cycle. You will continue your study of the angiosperm life cycle this week in the ANGIOSPERM GROWTH AND DEVELOPMENT laboratory.

MORPHOLOGY AND LIFE CYCLES OF VARIOUS PLANT GROUPS

❑   Work in groups of three. Pick up a tray of specimens which you will study. Lids of specimen containers have been labeled. Please keep each lid with its container to avoid confusion. Below is a list of the specimens you should have in your tray. Please return all the specimens to the tray at the end of the lab period.

moss gametophyte with sporophytes	pine ovulate cones (1st year)
fern leaf with sori	mature pine cones
fern gametophyte	pine seeds
fern gametophyte with sporophyte	Pine megagametophytes
Pine pollen cones	

❑   Use the appropriate Bioreview Sheet to help you study the life cycle of a moss and a fern.

Numbers on specimens' containers and numbers in parentheses in the lab text correspond to the numbers of structures shown on the Bioreview Sheet for the plant studied. The Bioreview Sheets have a more extensive terminology for structures than that used in the lab text. You need to know only terminology in the lab text.

Nonvascular Plants

These plants lack well-developed vascular tissues, and without xylem conduits must depend entirely on diffusion to transport water to their aerial structures. Consequently, nonvascular plants are relatively short or low profile plants.

PHYLUM BRYOPHYTA: MOSSES

❑   Observe the moss specimen.

The conspicuous leafy plant is the **gametophyte**, composed of several **gametophores** which may resemble vascular plant shoots. Both male and female gametophores may be present.   If male gametophores with antheridia are present, your lab instructor will indicate them to you.

A female gametophore can be recognized by having a **sporophyte** attached to it. A sporophyte consists of a slender stalk with a broader capsule at the tip; the base of the stalk is in an archegonium on the female gametophore.

A sporophyte originates from a zygote formed by syngamy of a sperm and an egg in an archegonium. The zygote develops into an embryo within the archegonium. As the embryo develops into a sporophyte the base of its stalk remains attached to the archegonium. Throughout its relatively short life the sporophyte remains attached to and somewhat dependent upon the gametophyte. The sporophyte may have a "hood" called a calyptra , which is a portion of the archegonium carried up by the sporophyte as it elongated.

If the calyptra is not present, you can see the details of the **capsule** which contains the **sporangium** in which spores are formed. Each capsule has a "cap," or operculum which is shed when the capsule is mature and ready to release spores.

Vascular Plants

The presence of xylem and phloem in vascular plants enables them to transport water and nutrients at a much greater rate than that observed in nonvascular plants. Vascular plants can be larger than mosses because photosynthetic structures can be farther away from water-absorbing structures. Furthermore, most vascular plants do not require the extremely moist environment needed by nonvascular plants. The conspicuous stage in the vascular plant life cycle is the **sporophyte**; the gametophyte is quite small and short-lived. A major evolutionary trend in the vascular plants is the reduction in size and complexity of the gametophyte, which you will see in this study.

The vascular plant sporophyte consists of two major morphological systems–a root system (generally underground) and a shoot system (generally aerial). A **shoot** is a stem with its various attached structures, such as leaves and buds.

PHYLUM PTEROPHYTA: FERNS

The term "ptero" of Pterophyta means wing, and refers to the relatively large leaves possessed by many plants in this group. Ancient fern-like plants were the first vascular plants to produce the "megaphyll," a relatively large, broad, flattened leaf with branching venation. The Pterophyta is one of two phyla of **seedless** vascular plants.

Most ferns are homosporous. A few species, collectively called "water ferns" because they are adapted for living in water all the time, are heterosporous. The specimens you will study are homosporous.

❑   Observe the specimens of the fern life cycle and consult the Bioreview Sheet "Fern Life Cycle."

   Note: On the Bioreview Sheet, sporophyte structures (of the dominant generation) are black; haploid structures (spores, gametophyte, gametes) are blue.

❑   Begin with the mature sporophyte (1) on the display bench.

The mature sporophyte of many fern species consists of an underground stem or a prostrate stem on the surface of the ground with true roots on its lower side and more or less vertical leaves on its upper side. Do you see any rolled-up, expanding leaves called "fiddleheads" (1, b)? Some fern species have vertical stems instead of horizontal ones giving them a tree form. On the fern displayed, clusters of **sporangia** called **sori** (singular: **sorus**) are on the "bottom" sides of mature leaves (2, 3).

❑   Observe sori on the sporophyte.

❑   Using a dissecting microscope, observe sori on the piece of fern leaf in the dish.

Each sporangium is somewhat spherical with a row of special cells called an "annulus" (4). As the sporangium dries, the annulus cells shrink until they create enough tension to rupture the sporangium, which releases the spores.

❑   Look for intact and broken sporangia and for spores. If conditions are right you may see the rupturing of sporangia.

A **spore** gives rise to a **gametophyte**, which when mature (6) is a thin green sheet-like structure with **rhizoids, antheridia** and **archegonia** on its underside.

❑   Observe mature gametophytes with the dissecting microscope.

These gametophytes were grown in a liquid culture medium. Naturally gametophytes develop on the soil surface and their rhizoids anchor them to the soil.

Multiflagellated sperm, released from an antheridium swim to archegonia, within which fertilization occurs. Each archegonium contains a single **egg** and thus may contain a single **zygote** (9, 10). Within the archegonium the zygote develops into an embryo which further develops into a young leafy sporophyte. The developing sporophyte is attached to and nutritionally dependent on the gametophyte until the sporophyte establishes a root system.

❑   Observe young sporophytes attached to gametophytes (12).

The gametophyte eventually dies and the sporophyte continues to develop as the dominant generation in the life cycle.

SEED PLANTS: GYMNOSPERMS AND ANGIOSPERMS

The seed plants made two major evolutionary advancements over other vascular plants in their adaptation to a terrestrial habitat. One was the production of **pollen** which, for most seed plants, eliminated the need for water to get sperm to eggs. The other was the production of the **seed**, in which the embryo can remain dormant and protected until environmental conditions are suitable for it to resume development.

Plants collectively called "gymnosperms" produce uncontained or "naked" seeds (gymno=naked; sperm=seed). Flowering plants, called angiosperms, produce seeds in a "vessel" called the **ovary** of the flower (angio=vessel; sperm=seed). In this study you will consider the reproductive structures of an angiosperm and pine, a member of the gymnosperm phylum Coniferophyta.

All the seed plants are heterosporous; they make two kinds of spores which give rise to two kinds of gametophytes (see Figure 6.2). The sperm-producing **microgametophytes** of seed plants are greatly reduced in size and complexity. A pine microgametophyte consists of only four cells, and one from an angiosperm consists of only two or three cells. Consequently antheridia do not occur in these gametophytes because their structure is too simple. A **pollen grain** consists of a **microgametophyte** contained in the cell wall of the **microspore** which gave rise to it.

The **megagametophyte** of a seed plant is not a free-living gametophyte like that of a fern. Instead it is retained within the **megasporangium**, which is usually called a nucellus (see Figure 6.2). The megasporangium (a sporophyte structure) is enclosed by a few layers of **integument**. Collectively the integument, megasporangium and megagametophyte compose an **ovule**. When an **egg** cell in a magagametophyte is fertilized, it develops into an **embryo** within the megagametophyte. A **seed** is a mature ovule, containing an embryo and a supply of stored nutrients, with the hardened integument forming the seed coat.

PHYLUM CONIFEROPHYTA (Example: Pine)

Conifers and some other gymnosperms produce reproductive structures called **cones**. The cone, in its simplest form, is a modified shoot consisting of a stem and special leaves called **sporophylls** which bear **sporangia**. A pine tree produces clusters of small, male (or pollinate) cones on certain parts of the tree, and larger female (or ovulate) cones on other parts.

❑   Consult the Bioreview Sheet, "Pine Life Cycle", observe a cluster of male cones (1) and examine a male cone (2) with the dissecting microscope. The numbers referred to below are on this sheet.

   NOTE: On the Bioreview Sheet, sporophyte structures (of the dominant generation) are black; haploid structures (spores, gametes, gametophyte) are red.

❑   Identify the cone bracts, (sporophylls) and their microsporangia, but do not mutilate the cone. Can you see pollen grains?

❑   Observe the prepared microscopic display of pine pollen.

❑   Examine a first year female cone (6-9).

   When pollination occurs in the spring the scales are widely separated and pollen grains can get between them. As the cones mature the scales grow together tightly. Pollen grains in the female cone enter each **ovule** through a small opening in its integument (7). At the time of pollination, megagametophytes are not yet formed in the ovules.

   In the year following pollination, the following events occur (steps 8, 9, 10, 11): 1) A **megagametophyte** with a few **archegonia** develops in each ovule; 2) Pollen grains germinate and pollen tubes grow through the megagametophyte to the archegonia; 3) **Sperm** are released from the pollen tube and the **egg** in each archegonium is fertilized (**syngamy**).

?   What is the difference between pollination and fertilization?

   Embryos develop during the second summer after pollination, but usually only one embryo in each megagametophyte survives. By late summer or early autumn of the second year, the mature ovulate cone spreads its bracts, releasing the seeds.

❑   Examine a mature ovulate cone. Look for seeds between the bracts, but do not remove them. If seeds are not present, look for two indentations near the base of each bract where the seeds were.

❑   Observe a complete seed.

   Several species of conifers (but not all) produce winged seeds. The wing is part of the seed coat.

❑   Obtain a pine megagametophyte. The large megagametophyte is from a pine species different from the one that produced the seeds you observed. With a razor blade, carefully cut the megagametophyte in half along the longer axis. The small, elongated structure in the middle of the gametophyte is the **sporophyte embryo** (13). Observe the embryo with a dissecting microscope.

   The embryo consists of a root-shoot axis with a few (more than three) cotyledons on the shoot end. The cotyledons are finger-like structures in a cluster. The megagametophyte is a source of nutrients for the embryo as it grows and develops into a sporophyte seedling. The cotyledons are embryonic leaves and become photosynthetic as the sporophyte develops (14).

❑   After you have examined the megagametophyte with its embryo, you may eat it and the others in the dish.

❏ Using what you know, place coniferophyta on the diagram on p. 109. Write the shared derived characters that you used to make that decision.

<u>PHYLUM</u> <u>ANTHOPHYTA</u> (Angiosperms/flowering plants)

The flower is a reproductive structure produced only by angiosperms. Cones of various gymnosperm groups are sometimes called "flowers" but they are not true flowers. A flower is a <u>determinate</u> <u>shoot</u> (a shoot with a limited capability for growth and development) with highly specialized **sporophylls**. Some flowers have only pollen-producing structures or only ovule-producing structures. A **perfect flower** has both pollen- and ovule-producing components (stamens and carpels, respectively). A **complete flower** has stamens, carpels and two additional sets of modified leaves called **petals** and **sepals**.

❏ Observe a cluster of *Gladiolus* flowers on a stem. Select a flower that is fully open, but not wilting.

❏ Position a razor blade at the side of the stem directly above where your selected flower is attached, and cut down along the side of the stem to remove the flower.

❏ Carefully remove the two small leaves surrounding the base of the flower to reveal the flower's **ovary**. Keep the leaves; you will use them later.

❏ Examine a complete flower. Use Figure 6.4 to identify the components of the flower.

A *Gladiolus* flower has three **petals** and three **sepals**, generally the same color, whose bases are fused to form a flared tube above the **ovary**.

? How many stamens are in the flower?_____

The **anther** of a **stamen** contains **microsporangia**, which produce **microspores**, which in turn become **pollen grains**. Do you see pollen on the surface of the anthers?

The "female" component of the flower is called a **carpel**, which consists of a **stigma**, a **style** and an **ovary**.

❏ Identify a stigma, the style and the ovary of a carpel.

A *Gladiolus* flower has three fused carpels; the stigmas are separate but the fused styles and ovaries appear as a single structure.

❏ Touch a stigma.

Is it sticky? When the carpel is mature and ready to receive pollen, the stigma becomes sticky to hold pollen grains.

❏ Remove the petals and sepals from one side of your flower. With a razor blade, cut longitudinally through the ovary to reveal the **ovules** (refer to Figure 6.4).
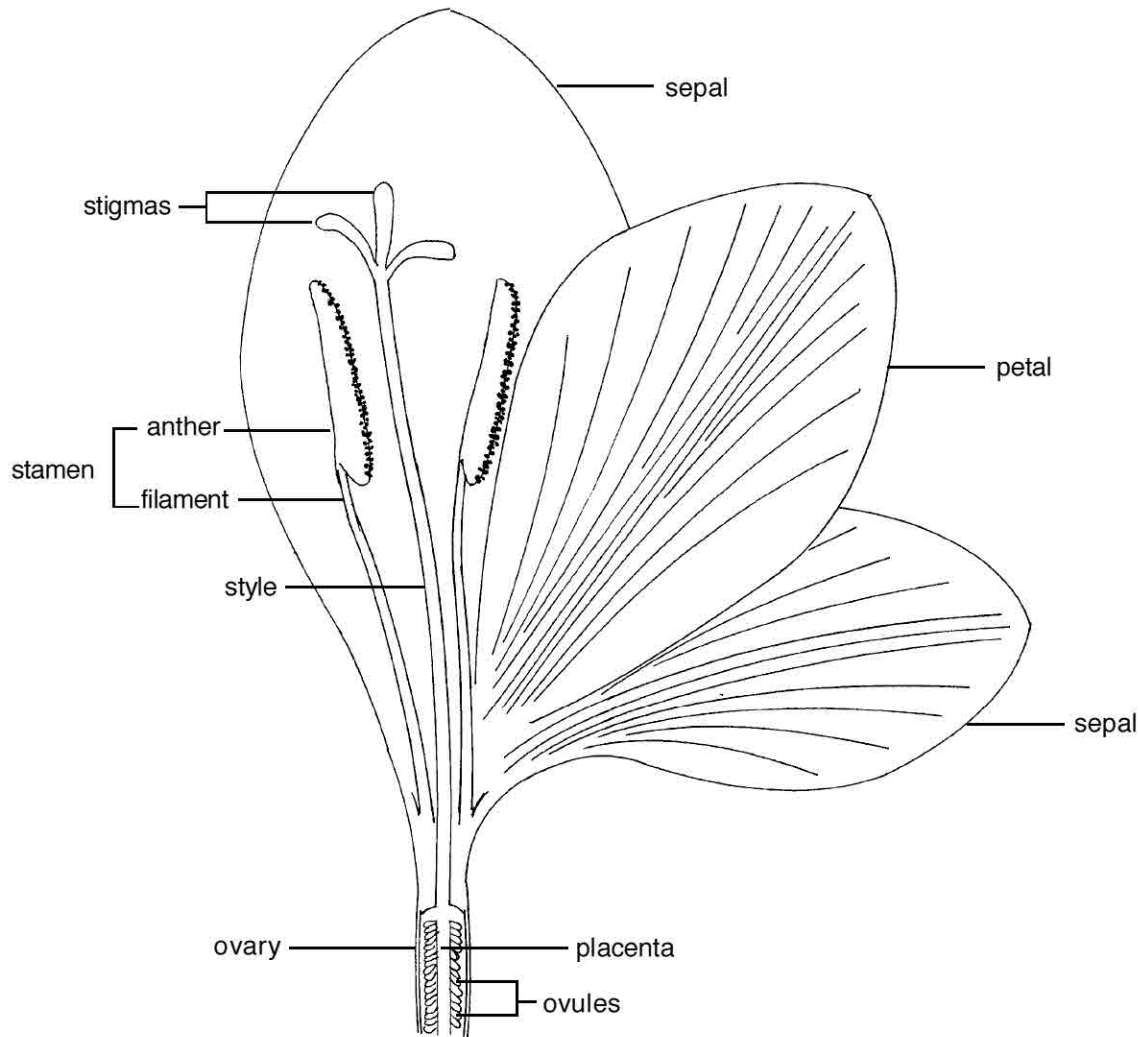
Figure 6.4. Diagram of a *Gladiolus* flower. One stamen, one sepal and two petals are not shown.

The ovules are contained in three chambers of the ovary. Each ovule is attached to a central column called the **placenta**, which provides water and nutrients for the growth of the ovule as it develops into a **seed**. When ovules becomes seeds, the ovary is the vessel containing the seeds of an angiosperm (*angeion*=vessel, *sperma*=seed).

Each ovule consists of an outer tissue called integument which contains a **megasporangium** (also called a nucellus), which, in turn, contains a **megagametophyte** (also called an embryo sac). See Figure 6.5.

The megagametophyte of an angiosperm is small and structurally simple. The typical example consists of six uninucleate cells and one larger, binucleate **central cell**. The **egg** is one of the uninucleate cells. The simplicity of the megagametophyte precludes it from having an archegonium.

Sexual reproduction in angiosperms involves **double fertilization**, a process found in no other group of plants except one minor gymnospermous group. The **microgametophyte** of a pollen grain consists of two cells: a tube cell and a generative cell. After a pollen grain is "accepted" by a stigma (i.e. pollination), the tube cell begins to grow into a tube which enters the stigma and continues to grow

through the length of the style into the ovary where it enters a megagametophyte in an ovule by growing through the micropyle, a pore in the integument. The pollen tube contains a tube nucleus and two sperm cells derived from the generative cell. After the tube cell enters the megagametophyte the two sperm cells are released. One sperm fuses with the egg to form a **zygote**; the other sperm fuses with the two nuclei of the central cell to form a **triploid nucleus** in a cell called the **primary endosperm cell** that gives rise to **endosperm**, the nutritive tissue of the angiosperm seed. See Campbell and Reece (2008) for more details on double fertilization.
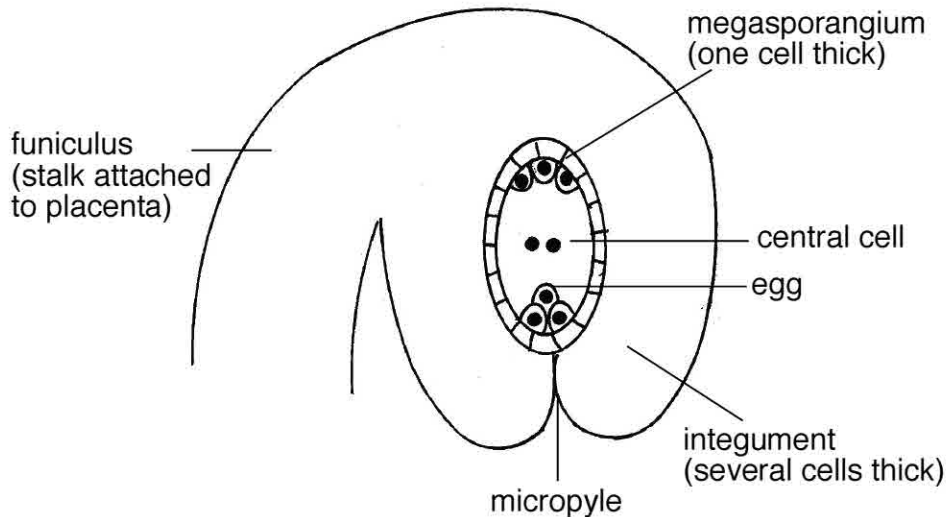
Figure 6.5. A simplified diagram of an angiosperm ovule. The nuclei of only the megagametophyte cells are shown.

The structure of the angiosperm seed and the functions of its components will be considered in the ANGIOSPERM GROWTH AND DEVELOPMENT laboratory next week.

Fruits are derived from flowers, thus they are unique to angiosperms. A fruit, in its simplest form, is a mature ovary. Many fruits, however, include other floral structures.

❑ At your convenience observe the displays of ANGIOSPERM SEXUAL REPRODUCTION and FRUITS in the hallway.

DIVERSITY IN THE ANGIOSPERMS

Monocots and Eudicots

❑ Review the number of stamens and stigmas you observed on your *Gladiolus* flower. Recall that the flower had three petals and three sepals.

Gladiolus sp. is a monocot, and like many different kinds of monocots, its flower components occur in threes or multiples of three.

❑ Observe a *Gladiolus* leaf and examine the arrangement of veins in the leaf.

The side-by-side ("parallel") venation pattern in the leaf is typical of many kinds of monocots and is generally not found in eudicots.

❑ Observe the eudicot specimens on display.

?   Can you distinguish petals from sepals in the flower? How many of each are present? _____ The petals may be separate or fused in the basal region.

Flower parts occurring in fours or fives (or multiples of those numbers) are typical of eudicot flowers; however, many kinds of eudicots have flower parts occurring in twos or threes.

❑   Compare the venation patterns in the leaves of monocot and eudicot specimens. How do they differ?

PLANT DIVERSITY ON THE CORNELL CAMPUS

The Cornell campus is rich with vegetation, but much of it is inconspicuous or less conspicuous in March than it is during the summer months. Nevertheless, in March (or even the middle of winter) one can observe many diverse specimens of the phyla **Anthophyta**, **Pterophyta** and **Cycadophyta** (cycads) in the BAILEY CONSERVATORY. This conservatory is the large glasshouse on the south side of the Plant Science Building. It is open from 8 a.m. to 4 p.m., Monday through Friday. You can enter it from a room connecting it to the Plant Science Building. The Conservatory contains plant specimens from all over the world. Most of the plants have labels giving names of their family, genus and species. The majority of the specimens are angiosperms, and many are flowering. The north room has a large collection of ferns. Several different species of cycads are in the south room.

Numerous members of the phylum **Coniferophyta** are scattered throughout the campus. A collection of specimens showing diversity in this phylum is located on the north side of Tower Road across from Stocking Hall.

Diversity in the gymnospermous phylum **Ginkgophyta** is minimal because this phylum has only one species, *Ginkgo biloba*. Nevertheless, several specimens of that species are present on campus and in Collegetown. Two trees are on the east side of Emerson Hall, and one is on the north side of Phillips Hall by Campus Road. In Collegetown, the south side of Dryden Road between Ithaca Road and Linden Avenue is lined with *Ginkgo biloba* trees. The trees are deciduous, so no leaves are present in March, but their knobby branches distinguish these trees from deciduous angiosperm trees.

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Scagel RF, Rouse GE, Schofield WB, Stein JR, Taylor TMC. 1966. An evolutionary survey of the plant kingdom. Belmont, CA: Wadsworth Publishing Co.

BioG 1108   **Name** _____

                                              Lab Instructor _____

SUMMARY WORKSHEET FOR PLANT LIFE CYCLES

1. All plants produce sporangia and have multicellular gametophytes. By completing the following table, you can compare the locations of sporangia on plants and the immediate environments of gametophytes in the different plant groups. If a plant group is heterosporous, give the locations of both microsporangia and megasporangia, and the "habitats" of both the microgametophyte and the megagametophyte.

    "Habitat" is the natural environment in which the gametophyte grows and develops to maturity. For example, if the gametophyte is free-living, its habitat would be soil, but if it remains dependent on the sporophyte, its habitat would be the sporophyte structure containing it.

Plant Group	Sporangium on/in what plant structure	"Habitat" of gametophyte
Bryophyta (mosses)		
Pterophyta (ferns)		
Coniferophyta (conifers)	micro- mega-	micro- mega-
Anthophyta (angiosperms)	micro- mega-	micro- mega-

2. All kinds of plants produce structures for dispersing their offspring. These structures are relatively resistant to harsh environmental conditions and capable of developing into new organisms. What is the main dispersal structure for each of these phyla?

    Bryophyta:_____

    Pterophyta:_____

    Coniferophyta: _____

    Anthophyta:_____

CHAPTER 7 - FUNCTIONAL ANALYSIS OF INVERTEBRATE DIVERSITY

LABORATORY SYNOPSIS

This two-day laboratory sequence provides a survey of several of the major invertebrate animal phyla. Through the study of prepared microscope slides, dissections, and demonstrations of living organisms, you will explore many aspects of invertebrate functional morphology, as well as obtain a sampling of the behavioral and physiological specializations that are found among members of the non-chordate animal phyla.

Below is a schedule for the two laboratory periods:

Day 1:
" Survey a majority of the invertebrate phyla, including the hydras, flatworms, roundworms, rotifers, and mollusks.

" Do a comparative dissection of two mollusks: the clam (a bivalve) and the squid (a cephalopod).

" Focus your attention on living and preserved specimens in order to learn the major characteristics and typical representatives of these phyla, as well as special adaptations they show for life in diverse habitats.

Day 2:
" Examine two additional phyla: the annelid worms and the arthropods (crustaceans, insects and their relatives).

" Dissect an earthworm (an annelid).

" Dissect a crayfish (a crustacean).

LABORATORY OBJECTIVES

At the end of these two laboratories you should&

1. know the principal anatomical characteristics that differentiate Cnidaria, Platyhelminthes, Rotifera, Nematoda, Mollusca, Annelida, Arthropoda, and be able to identify, from projected slides, photographs, or actual specimens, typical members of these groups.

2. have an understanding of the traditional grouping of animals into radiate, acoelomate, pseudocoelomate and coelomate phyla, and the alternate phylogenetic hypothesis of the lophotrochozoan and ecdysozoan clades within the protostomes, based on molecular systematics.

3. understand the major differences that suggest the division of the Bilateria into protostome and deuterostome lineages.

4. understand the following terms: invertebrate, radial and bilateral symmetry.

5. understand the structure and function of a gastrovascular cavity and a complete digestive tract, and be able to identify the principal structures in the digestive systems of the clam, the squid, the earthworm, and the crayfish.

6. know the difference between an open and closed circulatory system; know how the representative members of each phylum studied meet their own particular circulatory requirements.

7. know how representative members of each phylum studied meet their needs for gas exchange.

8.   understand the function and be able to identify the structures of the excretory and reproductive systems of the clam, the squid, the earthworm, and the crayfish.

9.   be able to explain different adaptations for locomotion as seen in the clam, squid, earthworm, and the crayfish.

10.   continue to develop good dissection skills.

READING ASSIGNMENTS (should be done <u>before</u> lab period).

For Day 1: In this chapter, pages 125–145; in *Biology* (Campbell and Reece 2008): pages 655–677.

For Day 2: In this chapter, pages 145–160; in *Biology* (Campbell and Reece 2008): pages 677–697.

Jon C. Glase

Revised June 2010
Mark A. Sarvary

PRE–LABORATORY ASSIGNMENT

To prepare you for this laboratory, based on the reading assignments listed above, complete the sections of Table 7.1 (page 162) that include the organisms you will be studying during each day's laboratory. On this chart you should indicate what structure(s) are used by members of each phylum for accomplishing the basic life functions. You should also identify the distinguishing characteristics of each phylum, list the major classes (either by common or scientific name), and make note of the representatives of each phylum that you will observe in the laboratory.

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

Day One

1.   Contrast radial and bilateral symmetry. Why has cephalization of the nervous system evolved with bilateral symmetry?

2.   What is a body cavity, and why is it beneficial to an animal?

3.   What characteristics distinguish protostome from deuterostome animals?

4.   What is a gastrovascular cavity? In which groups is a gastrovascular cavity found?

5.   What are the three major regions of the molluscan body plan?

6.   How do the lifestyles of clams and squid differ? How is this reflected in their functional morphology?

Day Two

7.   What characteristic led biologists to hypothesize that annelids and arthropods are closely related? In which clades are these two phyla placed based on molecular data?

8.   Contrast the movement of an earthworm with that of a nematode (roundworm).

9.   Contrast the circulatory system of an earthworm with that of a crayfish.

10.   Describe the reproduction of earthworms and crayfish.

DAY 1

INTRODUCTION

This laboratory introduces you to representatives of phyla that include over 95% of the described animal species. These species, collectively known as the invertebrates, include all animals except those in the vertebrate subphylum of the phylum Chordata. As their name implies, the invertebrates lack a backbone. Unlike the vertebrates, whose body design is constrained by its endoskeletal system, the invertebrates exhibit an incredible diversity of body form and organization. This diversity of form leads to comparable diversity in life history, as well as morphological specializations for nutrient procurement, digestion, movement, respiration, excretion, neural integration, reproduction, and behavior.

It is not known with certainty why invertebrates are so diverse, but one commonly held opinion is that small size is the key to their evolutionary success. As a result of small size, the portions of the environment that they require for food, shelter, reproduction, etc. are also correspondingly small. These small **niches** allow for many little domains where specialists can peacefully coexist. For example, the army ants of South America play host to several species of parasitic mites, small members of the phylum Arthropoda. One kind of mite is specialized to live only on the mouthparts of the ant, where it feeds on scraps of food, while another is found only on the ant's hind foot, where it sucks hemolymph for a living.

Invertebrates also rule the animal world by virtue of sheer body mass. For example, in a typical tropical rain forest, each hectare (or 2.5 acres) contains a few dozen individual birds and mammals but well over one <u>billion</u> invertebrates. There are about 200 kilograms dry weight of animal tissue in that hectare, of which 93% consists of invertebrates. So when you walk through a tropical forest, or most other terrestrial habitats for that matter, or snorkel above a coral reef or some other aquatic environment, vertebrates may be the animals that catch your eye, but you are actually visiting a primarily invertebrate world.

Despite their overwhelming abundance in the environment, much remains to be learned about most invertebrates. When you scoop up a shovel-full of earth virtually anywhere on earth except the most barren deserts, you will find thousands of invertebrates ranging in size from the clearly visible to the microscopic. The biology of most of the species you hold is unknown; we have only the vaguest ideas about what they eat, what eats them, and the details of their life cycle. We know very little about their biochemistry and genetics. Still, some groups of invertebrates and certain aspects of their lives are reasonably well known, and it is on these groups that we shall focus our survey of the invertebrate animals.

ORGANIZING THE INVERTEBRATE PHYLA

We will survey seven phyla in our consideration of invertebrate diversity. The proposed evolutionary relationships among these taxa can be represented in a phylogenetic tree. See Figure 32.10 on page 662 in *Biology* (Campbell and Reece 2008), which depicts the traditional view of phylogenetic relationships based on body plan and embryology. We will follow that organization in our survey of invertebrate phyla. But also note Figure 32.11 on page 663, which depicts an alternate hypothesis of animal phylogeny based on molecular systematics. Molecular data provide a new set of characters in determining branch points and monophyletic clades in the cladistic analysis of evolutionary relationships.

The traditional and molecular phylogenetic trees agree on the deepest branch points of animal phylogeny. All animal phyla, except Porifera (the sponges), are placed in a group called the Eumetazoa ("true animals") and are assumed to have the same evolutionary origin. The phylum Cnidaria, placed in a subdivision of the Eumetazoa called the Radiata, is set off from the main stem of animal evolution, based on its possession of **radial symmetry**. The remaining eumetazoan phyla, placed in a subdivision called the Bilateria, all show **bilateral symmetry** (although some are secondarily radial as adults), possess ectodermal, mesodermal, and endodermal tissue layers, and show **organ level** anatomical organization.

The symmetry of an animal's body is most often related to its <u>pattern of locomotion</u> (Figure 7.1). <u>Sessile</u> or sedentary animals tend to exhibit radial symmetry. The body of these animals is generally in the form of a cylinder with a basal end that is attached to the substrate, a free oral (or upper) end, and other body parts such as tentacles or arms arranged radially around the main body axis. <u>Motile</u> animals, in contrast, usually pass through the environment with the same end of their body forward at all times. Their bodies are elongated through the axis of travel, with a tendency toward **cephalization**, the concentration of sense organs and nervous tissue within a head (anterior) region. This type of body organization is known as bilateral symmetry—a condition where the body can be divided along a longitudinal plane into right and left halves that are mirror images of each other.
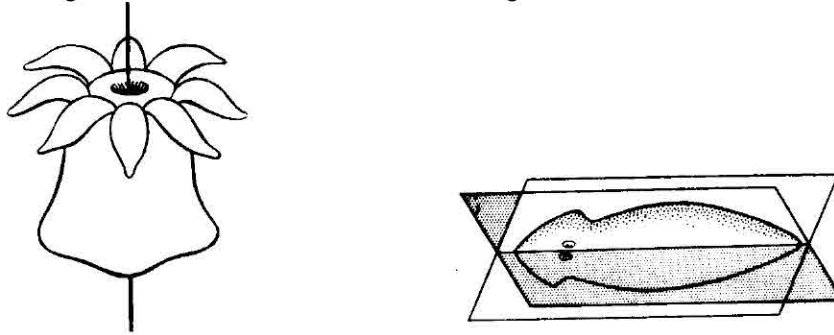
Figure 7.1. Examples of radial and bilateral symmetry among invertebrates (Buchsbaum and others 1987).

An important criterion that is used in the traditional, body-plan phylogeny to organize the Bilateria depends on the presence or absence of a **coelom**, or true body cavity. The coelomic cavity is located between the external surface of the digestive tract and the inner surface of the body wall, and provides a major benefit by allowing the organism to move through the environment independently of motions being generated by the activity of its gut. A fluid-filled body cavity can also serve as a **hydrostatic skeleton**, useful in certain types of locomotion. The Bilateria phyla have traditionally been separated on the basis of coelomic development. Some phyla are **acoelomate** (having no coelom), others are **pseudocoelomate** (having a cavity that is only partially lined with mesoderm), and still others are **coelomate** (having a complete, mesodermally-lined coelom).

The coelomate phyla have been further divided into the Protostomia and Deuterostomia, based primarily on embryological and developmental characteristics. A major difference between the members in these two lineages is the origin of the mouth. Embryos in both groups form a pore (opening) in one end early in their development. In a protostome embryo, this pore becomes the mouth of the developing organism (proto = <u>first</u>; stome = <u>mouth</u>). In a deuterostome embryo, the first pore becomes the <u>anus</u>, and a second pore, formed later in embryonic development, becomes the mouth (deutero = second; stome = mouth). Protostomes and deuterostomes also differ in the patterns of the first cell divisions and the developmental capabilities of the first few cells formed in their embryos.

Both the traditional and molecular phylogenies recognize the deuterostomes as a monophyletic group. The molecular-based phylogenetic tree, however, places the acoelomate and pseudocoelomate phyla within the Protostomia. Molecular systematics also distinguishes two distinct clades—the Lophotrochozoa and Ecdysozoa—within the Protostomia. The traditional tree proposes a close evolutionary link between annelids and arthropods, primarily based on body segmentation. In the molecular hypothesis, annelids cluster with the lophotrochozoan phyla, and arthropods are placed in the ecdysozoan clade. Although our study is organized according to the traditional tree, we will also reference the molecular phylogeny. Reconstructing the evolutionary history of the animals continues to be an area of active research.

A pattern of increasing complexity in body organization can be traced through a phylogenetic tree of the animals. Within each phylum, however, there still exists a surprising variety of form, structure, and life style, all reflecting the evolutionary history of related organisms that were exposed to different habitats and therefore different selective pressures. The phylum Mollusca, for example, includes animals that are filter feeders, rasping herbivores, or active predators; ones that "breathe" with gills or lungs; some that are sessile, creeping, or fast-swimmers; and representatives that are exclusively marine, freshwater, or terrestrial inhabitants. During our study of the increasing specialization and complexity among the invertebrates, remember that, no matter where they occur on a phylogenetic tree, these animals are all very well adapted for successfully dealing with their environment. Success is not necessarily measured by the elaboration of body form, or even by numbers of species or individuals. Success, from an evolutionary perspective, rests on effective reproduction and the resultant continuation of the species.

SECTION RADIATA: PHYLUM CNIDARIA

The phylum Cnidaria is an aquatic (mostly marine) group of organisms that includes the hydra, jellyfish, sea anemones and corals. The internal anatomy of *Hydra* is representative of the phylum, and is shown in Figure 7.2

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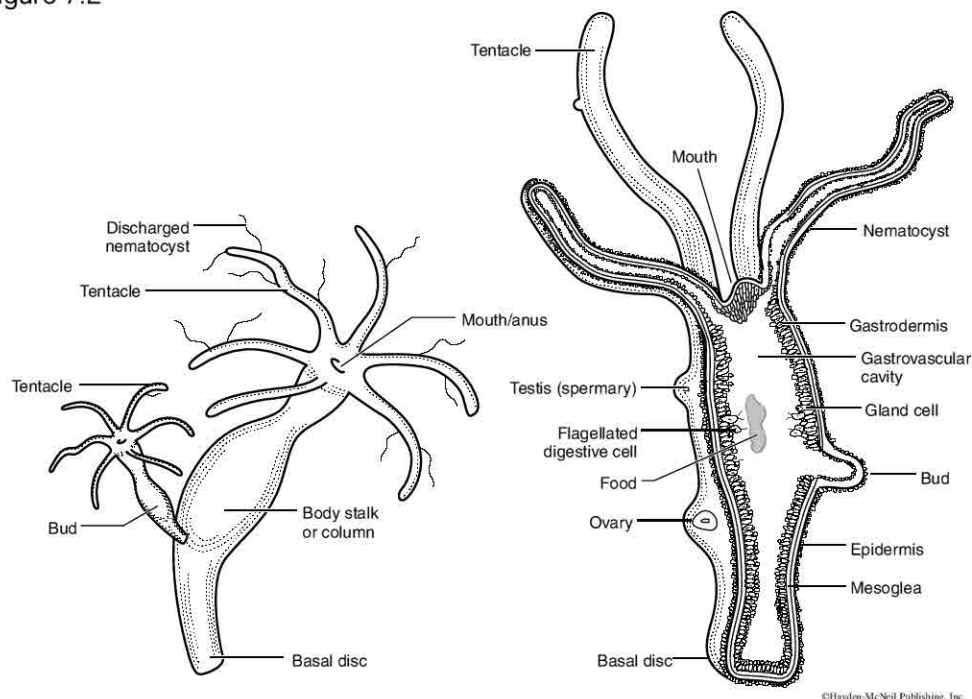
Figure 7.2. Longitudinal section through *Hydra* (Cnidaria, Hydrozoa).

Cnidarians are characterized by having **radial symmetry**, a **gastrovascular cavity**, and **tentacles** bearing stinging **nematocysts**, which are used for defense and prey capture. Their sac-like bodies consist of two basic cell layers, an **epidermis** with some cells specialized as sensory-nerve cells, gland cells, stinging cells, and epithelial-muscular cells; and an inner **gastrodermis** made up of nutritive-muscular cells and enzymatic gland cells. Between these two cell layers is a jelly-like **mesoglea** layer with scattered amoeboid or fibrous cells. The life cycle of most cnidarians includes an alternation between two body forms: a relatively stationary **polyp** stage (like a hydra) that reproduces asexually, and a free-floating **medusa** stage (like a jellyfish) that reproduces sexually.

Class Hydrozoa - *Hydra*

❑ Observe living hydra with the dissecting microscope. These animals are capable of great extension and contraction. Although usually sessile, hydra can glide on its base, float by means of a gas bubble secreted under the **basal disc**, and somersault. Its body is essentially a hollow cylinder, differentiated at the basal end as an adhesive disc for attachment to the substrate or plants and at the oral end by a **mouth**, which rests on a conical elevation surrounded by a ring of **tentacles**. These tentacles contain stinging cells called nematocysts, important in the capture of small crustaceans and insects.

Hydra is unusual among the Hydrozoa in having only a polyp stage. A rounded projection, or bud, consisting of all cell layers, develops along the body wall of the parent animal. Tentacles appear on the distal end of the bud, and soon thereafter the new small hydra separates from the parent.

❑ Can you observe any buds on the *Hydra* in the live culture. Asexual reproduction may occur at anytime.

❑ Observe the demonstration of discharged **nematocysts** that your lab instructor has prepared by adding some stain to a living hydra on a depression slide. Notice the highly coiled strands and barbed ends of the nematocysts.

After the stinging cells have immobilized the prey, tentacles stuff it into the gastrovascular cavity. Gland cells then secrete digestive enzymes and mucus. Flagellated gastrodermal cells are phagocytic and can engulf partly digested food particles. Undigested material is ejected back through the mouth. The mouth thus serves as both an entrance for food and an exit for wastes, a fundamental characteristic of all gastrovascular cavities.

ACOELOMATES: PHYLUM PLATYHELMINTHES (Lophotrochozoan Clade)

The phylum Platyhelminthes (*platy*, flat; *helmin*, worm; Gk.) includes the free-living planarians and the parasitic flukes and tapeworms. The flatworms are characterized by **bilateral symmetry**, three well-developed tissue layers (**ectoderm**, **mesoderm**, **endoderm**), and a **gastrovascular cavity** (Figure 7.3 a, b, c.). In the Platyhelminthes, the mesoderm develops into muscles and other structures that make possible an increasing complexity in animal activities. The flatworms are **acoelomate**; there are no spaces within this mesodermal layer, and thus there is no mesodermally-lined body cavity, or coelom. This group has an extensively branched, tubular excretory system that removes nitrogenous waste products from the body fluids. It is driven by ciliated cells called **flame cells**. Gas exchange and circulation of materials are by simple diffusion.

The molecular-based phylogeny places the Platyhelminthes within the lophotrochozoan clade, implying that flatworms are not primitive "acoelomate" animals but that their body plan became simplified by the loss of the coelom later in evolution.

Class Turbellaria - *Planaria*

❑ Observe the slide labeled 'Planaria, double mount' with the compound microscope. One of the planaria on this slide has been stained so that the gastrovascular cavity is quite dark. The other specimen is not stained. In the center of the body is a clear, oval region, the pharyngeal chamber, in which is situated the muscular **pharynx**. The pharynx, which opens at the **mouth**, is protruded from the ventral side of the body when the animal feeds. The anterior end of the pharynx opens into the **gastrovascular cavity**, which divides into three main trunks, a median anterior trunk which extends forward to the eyespots, and two posterior trunks which run on either side of the pharyngeal chamber to the posterior end of the animal.

?   What might be the advantage gained by having these extensively branched gastrovascular trunks?

❑   Label the indicated structures of the planarians illustrated in Figure 7.3.

❑   Examine the live planarian, *Dugesia tigrina*, under the dissecting microscope. The peculiar gliding form of locomotion is due to the movement of cilia on the ventral surface of the animal. The anterior end of the animal is expanded into **auricles** on which are located touch and chemoreceptors. The **eyespot** consists of a clear area where the sensory cells are located and a crescent-shaped mass of black, densely packed granules, whose function apparently is to concentrate light upon the sensory cells. The eye has no lens and thus cannot perceive objects. It is, however, quite good at distinguishing different light intensities.
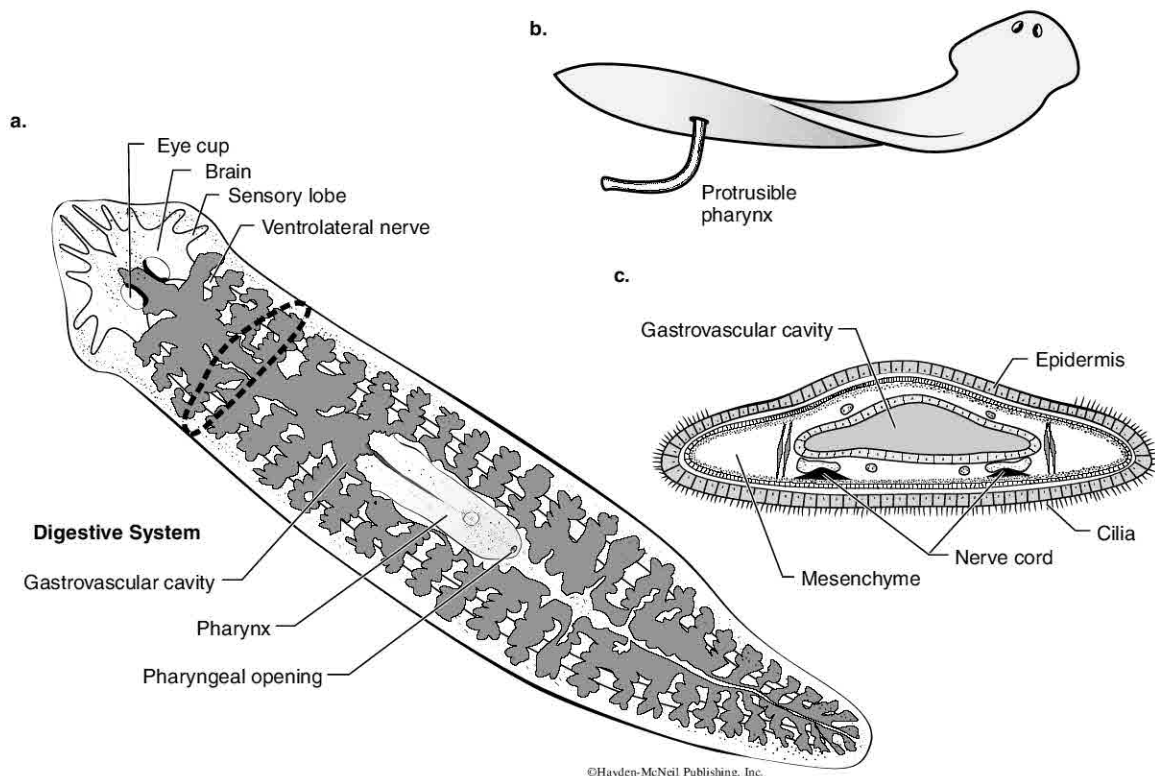
Figure 7.3. Diagrams of a planarian (Platyhelminthes, Turbellaria) showing (a) digestive cavity, (b) mouth and pharynx, and (c) a cross-sectional view of the generalized flatworm body plan (Buchsbaum and others 1987).

PSEUDOCOELOMATES

Some invertebrates are **bilaterally symmetrical** and possess a **pseudocoel**. A pseudocoel is a fluid-filled body cavity that separates the gut from the body wall, but that is not completely lined by mesoderm. The two phyla that we will consider are Nematoda, including the ubiquitous nematodes, or roundworms, and Rotifera, including microscopic, usually free-living aquatic animals with a crown of beating cilia at the anterior end. Most of the organisms in these groups are small, often microscopic, and have in common a **complete simple digestive tract**: mouth, intestine, and anus; a nonliving, protective body covering called the **cuticle**, and a **pseudocoel** in which the organs of reproduction and the flame cell-based excretory system are located. Gas exchange and circulation of materials are by simple diffusion.

The molecular-based phylogeny does not cluster these two phyla together, but groups the Rotifera within the Lophotrochozoa and the Nematoda within the Ecdysozoa.

PHYLUM NEMATODA - the roundworms (Ecdysozoan Clade)

❑   Observe the nematodes called vinegar eels (they live in vinegar!) with a compound microscope (under low and then high power). Note the thrashing movement of the worms.

Nematodes are usually cylindrical and tapered at both ends. A transparent acellular **cuticle** covers the body. The distinctive cylindrical shape of nematodes results from the high internal pressure of fluids within the body cavity (pseudocoelom). This fluid-filled cavity acts as a **hydrostatic skeleton** against which the muscles can work to create movement. Four rigid projections of the body epidermal layer, called cords, divide the one cell-thick longitudinal muscle layer into four functional fields. The lateral cords are larger than the dorsal and ventral cords, and may be seen externally as **lateral lines**. There are no circular muscles. The alternate contraction of different longitudinal muscle fields in conjunction with the high hydrostatic internal pressure creates the characteristic whiplike or thrashing movement of nematodes.

PHYLUM ROTIFERA - the rotifers (Lophotrochozoan Clade)

❑   Observe rotifers with a compound microscope (under low and then high power)**.**

The cuticle-covered body of a rotifer is transparent and cylindrical. This cuticle may occur in sections so that the body may telescope. Notice the movement of a rotifer, how it can twist, bend, flex, shorten, and lengthen. The muscles, which are usually in separate bundles rather than in the regular circular and longitudinal muscle layers of most other worms, traverse the pseudocoelom to allow for this motion. Rotifers can be observed walking by means of the foot, a structure that emerges from the posterior tapering end of the body. Rotifers also swim with the aid of currents set up by the rotating wheel of cilia. This wheel of cilia is also the principal food gathering device, creating a current of water that draws food particles into the mouth. The mouth leads into the muscular pharynx, which contains a cuticularized grinding organ called a mastax. Muscular activity of the pharynx causes the mastax to grind and chew, which you should easily be able to observe under high power. Early microscopists mistakenly thought this active area of the rotifer was its heart.
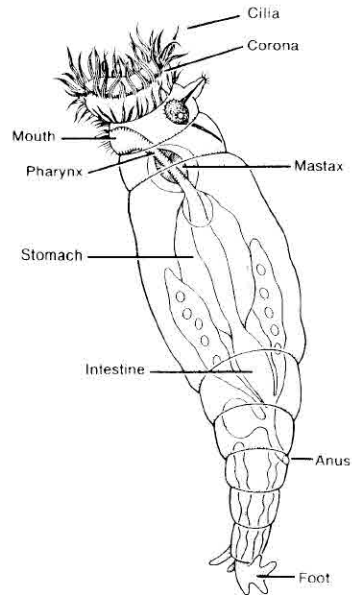
Figure 7.4. Characteristic features of a typical rotifer (Abramoff and Thomson 1982).

❑ Identify the **foot**, **wheel of cilia** or **corona**, the **mastax**, **pharynx**, and **stomach** on the rotifers (see Figure 7.4).

PROTOSTOMIA: PHYLUM MOLLUSCA (Lophotrochozoan Clade)

A generalized molluscan body plan includes three main regions: foot, visceral mass, and mantle. The **foot region** contains sensory organs and the muscular locomotory foot. The **visceral mass** contains the digestive, excretory, and reproductive organs. The **mantle** forms a fleshy cover over the visceral mass, secretes the **shell** (if there is one), contains the gills, and functions in respiration. Mollusks like the gastropods also have a **radula**, a rasping organ with transverse rows of chitinous teeth that scrapes up food particles. Filter feeders like the bivalves have a rotating crystalline **style** within the stomach which helps to pull in the mucus-food complex and releases digestive enzymes. Mucus-coated **cilia** cover most external and internal surfaces, aiding in the aeration of the gills, filter feeding, locomotion, and moving materials through the gut, kidneys, and reproductive ducts. The coelom in mollusks is found only in the region surrounding the heart. The circulatory system is **open** (except in the cephalopods), which means that the blood pools in open sinuses and bathes the tissues directly between circuits through the pumping heart and a very few vessels.

All major classes of mollusks reflect the elaboration or suppression of one or more of the three main body regions. We shall examine only two of the three most familiar classes (Figure 7.5): the Bivalvia (clams, oysters, scallops, mussels) and Cephalopoda (squids, octopuses). Molecular-based phylogeny places the mollusks within the clade Lophotrochozoa.

GASTROPODA   **BIVALVIA**   **CEPHALOPODA**
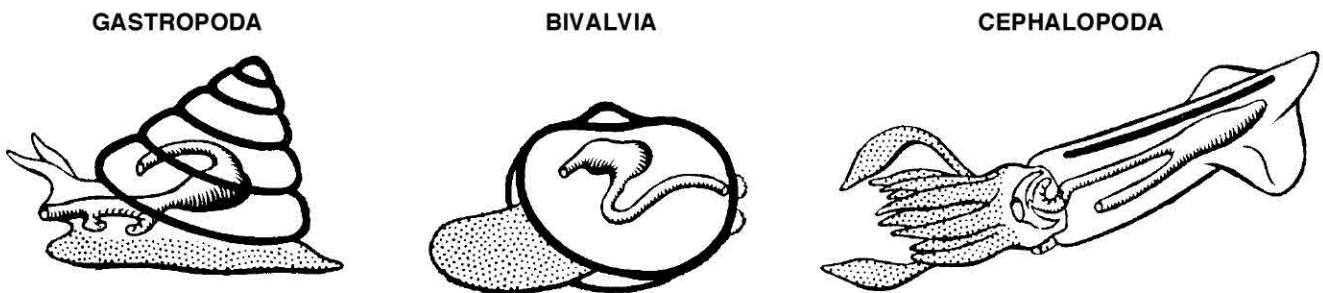
Figure 7.5. Three important classes of mollusks (Buchsbaum and others 1987).

Class Bivalvia - the clams, mussels, and their relatives

The "two-valved" mollusks have a laterally compressed body covered by a pair of shells secreted by the two-lobed mantle. Sensory tentacles, a well-developed head, and a radula are lacking. Incurrent and excurrent siphons channel water through the mantle cavity where the mucus-coated cilia covering the gills separate food particles from unwanted material such as sand and mud. A crystalline style in the stomach then winds in the food-laden strings of mucus, which are fed into the mouth by the labial palpi.

Class Cephalopoda - squid, octopuses, and their relatives

The cephalopods are among the most highly developed invertebrates. They have adapted the molluscan body plan to a rapid swimming, predatory life style. The nautiluses retain a large, coiled shell, but in squids and octopuses, the shell has been reduced to a small, underlined structure. The foot has been modified into arms and tentacles, as well as a muscular tube, the **funnel**, through which water from the mantle cavity is expelled to provide the propulsive power for swimming. The circulatory system is **closed**, with separate hearts pumping deoxygenated blood to the gills and oxygenated blood throughout the body. The nervous system of cephalopods is highly developed, possessing such advanced sensory organs as image-forming eyes, complete with lens, iris, retina, and pupil.

Comparative Dissection of the Clam and Squid — Instructions

During this dissection you will work in groups of two. In each section of the following procedure you alternate between dissection of clam and squid. Two students will be responsible for dissection of one organism, while the other two carefully observe the dissection, and then your roles will be reversed for the other organism. The objective of this comparative approach is to try to understand the differences and similarities seen in these two mollusks in terms of their life history strategies, as presented below. You will be responsible for knowing the structures discussed in the lab text for *both* organisms. At each break point, accomplish the activities and answer all questions before you continue.

Note: in both the clam and squid dissection, you will need to carefully use the binocular, stereoscopic "dissection" microscope to be able to clearly see all structures being considered. Each pair of students should obtain an instrument from the microscope cabinet and set it up for use now.

Life History Information

The Clam (*Mercinaria mercinaria*)

This species, known by the common names quahog, hard-shelled clam, or littleneck clam, inhabits the shallow, protected waters of the Atlantic coast. *Mercinaria* is a sedentary, bottom-dwelling organism, usually found in the lower intertidal areas buried in sand or mud with only its siphons exposed. It uses its foot to crawl and bury itself. The clam filter-feeds on plankton and organic material by moving water in its incurrent siphon, across its gills, and expelling it via its excurrent siphon. When disturbed, clams withdraw completely into their shells. The clam has a limited social life. Although large numbers congregate in suitable habitats, little interaction occurs between individuals. Reproduction is initiated by water temperature or salinity changes. Gametes are discharged from the animal by the excurrent siphon into the surrounding water and fertilization is external. Larvae may remain free-swimming for several months before settling and transforming into adults.

The lifestyle of the clam has some important implications for its ecology and survival. First, since its gills are so vital for both gas exchange and collecting food, the water entering the mantle cavity must be fairly free of sediment or the clam will suffocate and/or starve to death. Any disturbance that introduces a lot of sediment will destroy clam beds. On the other hand, if the water is totally free of organic particulates, the clam will starve.

Also, if clams are to be consumed by humans, it is important to monitor toxic particulates that may be present in their water. Any toxins, such as those from a "red tide", will be filtered out and accumulate in the clam to high, possibly dangerous levels. Low concentrations in the environment will be biologically magnified in the clam's tissues. This is also true for disease organisms and viruses such as those causing hepatitis B.

<u>The</u> <u>Squid</u> (*Loligo pealei*)

Squid are pelagic animals, found actively swimming in the open waters of the ocean. This species inhabits coastal regions of the Atlantic ocean from Maine to South Carolina. Squid are predatory on small fish and zooplankton. Their two tentacles are used to snatch prey; the eight arms hold it while feeding. To avoid their own predators, squid rely on rapid locomotion, evasive behavior, and discharge of ink from their ink gland.

Squid have a highly complex social life. They live in schools of from ten to several hundred. Individuals can communicate "mood" by rapid color change via chromatophores in their skin. Many species have a complex courtship behavior. During copulation the male uses a specially modified arm to insert a spermatophore (sperm package) into the mantle cavity of the female. Fertilization is internal. Fertilized eggs then receive a protective shell and are deposited in groups on the ocean bottom by the female. Squid embryos develop directly into adults with no larval stage.

External Morphology

<u>Clam</u>

❏ Obtain a live clam and note the animal is wholly or almost wholly enclosed in a bivalved shell, which constitutes its <u>exoskeleton</u>. Orient the shell (as in Figure 7.6) so that the swollen projection, the **umbo**, or beak, is on top and to the left (anterior end) of the clam. The region where the two valves (shells) are united is dorsal. In many marine bivalves, one or two tubes, the **siphons**, project from the posterior end. Dorsal, ventral, anterior, and posterior ends being thus defined, the valves are seen to cover the right and left sides of the animal. Is the animal bilaterally symmetrical?

❏ Examine empty valves of *Mercinaria*. Study the <u>external surface</u> first. The dorsal margin of each valve is hinged to the other by a tough elastic band, called the **hinge ligament**. This ligament causes the valves to open so that empty shells gape. The concentric rings on the shells of mollusks are referred to as growth rings. Which part of the shell do you suppose forms first?

❏ Examine the smooth, pearly, and concave <u>internal surface</u> of the empty shells. Notice the many tiny teeth along the free ventral edge of the valves and the larger interlocking teeth just anterior and posterior to the hinge ligament. How might these sets of teeth function? Test your hypothesis.

❏ Continue your examination of the inner surface of the shells. The faint line that follows along the margin of the shell marks the edge of the attached **mantle** tissue inside the shell. At the posterior end of the shell, this line furrows inward. The incurrent and the excurrent openings (**siphons**), which carry water to and from the mantle cavity, originate in this region. The scars of the **anterior** and **posterior adductor muscles**, the powerful muscles that close the valves, are found in each side in a large oval impression.

<u>Squid</u>

You will dissect a doubly-injected, preserved specimen of *Loligo pealei*. The injected latex will allow you to better understand the organization of the circulatory system, and the preservative has made the body and organs much more rigid and durable for dissection.

The body of the squid has elongated along the dorsal-ventral axis such that the foot (modified to form the arms, tentacles, and funnel) and the head, which have a ventral position in gastropods and bivalves, are now located at the functional anterior end of the animal. For purposes of your orientation, consider the flattened side with the projecting funnel as ventral. You will perform most of your dissection through this ventral surface, with the tentacles and head facing toward you.

❑ The modified foot has been drawn out into ten sucker-bearing **arms**; four pairs are true arms of about equal length, and one pair is the expandable **tentacles**, which can shoot forward to seize prey and then retract back to the mouth. The eight arms hold the prey securely while it is killed by an injection of toxin from salivary glands located near the mouth. Look at the arrangement of **suckers** on the arms. Remove a sucker and study it under the dissecting microscope. Note the muscular stalk and the toothed chitinous ring which supports the edge of the cup.

❑ Turn back the arms and tentacles and note the **mouth**, surrounded by muscular membranes extending from the bases of the arms. The outer, seven-lobed **buccal membrane** has suckers on its inner surface. The inner membrane surrounds the mouth opening through which the black chitinous **beaks** are visible.

❑ Use the fine scissors to remove the cornea (yellowish-clear tissue) of one eye and expose the spherical lens. The cornea, iris, pupil, and lens can be identified in the laterally-placed pair of **eyes**. Next remove the lens to expose the darkly colored retina below. The eyes of squid are almost completely analogous in function to the same structures in vertebrates, although the manner in which they develop embryologically is quite different.

❑ The majority of the body is covered by the fleshy, muscular **mantle**, which is shaped like a cone around the visceral mass and extends as two lateral **fins** at the apex. These fins are used for stabilizing and slow swimming. The free anterior edge of the mantle, called the **collar**, is divided into three scallops; the two lateral ones mark supporting cartilaginous ridges and the dorsal projection marks the anterior end of the **pen**, the internal shell of the squid.

❑ The muscular **funnel**, derived from the foot, extends from beneath the mantle on the ventral side. When the muscular mantle expands, water enters the mantle cavity around the collar. When the mantle forcibly contracts, the collar seals around the head, and water is forced out through the funnel, creating jet-propulsive motion. The funnel can be pointed anteriorly or posteriorly for directing movement. The constant stream of water through the mantle cavity also keeps the gills well aerated.

BREAK 1 — External Morphology

❑ Review what you have learned about both organisms.

❑ Orient both organisms so that their dorsal surfaces are uppermost and their anterior ends are facing you. Compare the three major molluscan body regions in both specimens.

❑ Consider the general body shape and external morphology of these two mollusks. Discuss differences that you see in terms of adaptations for&

	Clam	*Squid*
locomotion:		
food procurement:		
protection from predators:		

....

Gross Internal Morphology

<u>Clam</u>

❑ Orient the live clam with the anterior end to the left. The valves were opened by severing the anterior and posterior adductor muscles with a knife. The membranous **mantle** adheres closely to the inner surface of each valve. Note: the mantle was carefully freed from the left valve when the clam was opened. What are the functions of the mantle?
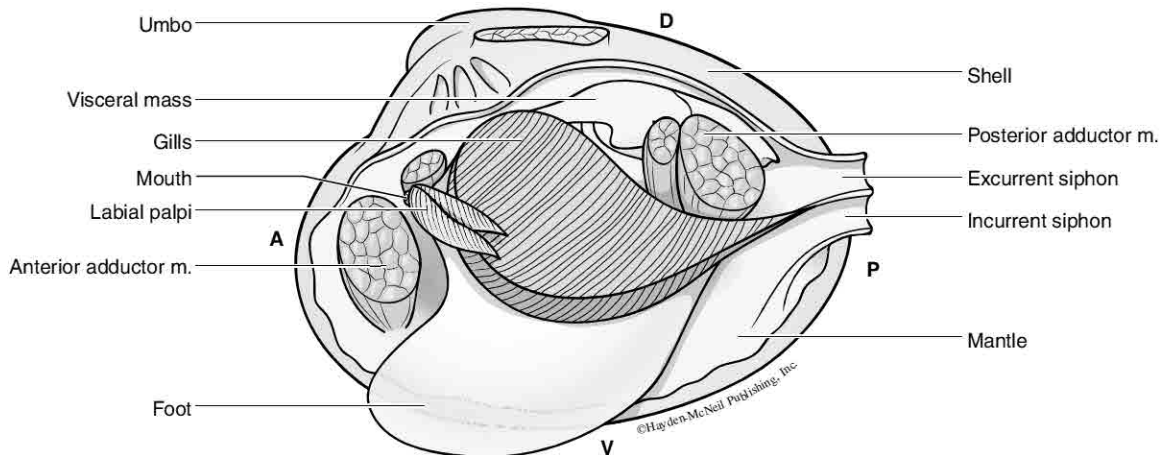
Figure 7.6. Internal anatomy of a clam with left valve and mantle removed (Mollusca, Bivalvia) (Buchsbaum and others 1987).

❑ Note the mantle lobes form the **siphons** at the posterior end of the animal. The siphons may be difficult to locate because the mantle may be torn in opening the clam. To see the siphons, bring the two flaps of the mantle together and look at this from the posterior end. The more dorsal and smaller opening is the **excurrent** siphon, serving for egress of water; the ventral and larger opening is the **incurrent** siphon, used for intake of water. Both openings have dark-colored lips, those of the incurrent siphon possess many, small nipple-like protuberances, called papillae. For what function may these papillae serve?

❑   Lift the mantle lobe and observe the body of the animal as a soft, elongated mass, flattened laterally, and ending ventrally in a keel-like structure, the **foot** (Figure 7.6). The foot is hard and firm due to its muscularity. The softer, stouter part of the clam dorsal to the foot is the **visceral mass**, which contains the internal organs. On each side partly covering the visceral mass are two long thin flaps, the **gills** (ctenidia). The most dorsal portion of the animal consists of a thin membrane enclosing a dark, **pericardial cavity**. Locate this cavity before proceeding.

❑   Note that the foot is directed toward the anterior end, the end opposite the siphons. Is a head present? Near the anterior end of the visceral mass on either side are a pair of flaps, the **labial palpi** (**palpus**, sing.). The posterior ends of the palpi are free; the anterior ends are connected to each other and to the mantle. Follow the two palpi of one side anteriorly as they narrow to mere folds, the upper and lower lips. Between these lips in the median line, directly behind the anterior adductor muscle in clams, is found an elongated slit, the **mouth** (Figure 7.6). What is the purpose of the palpi? (Hint: The whole inner surface of the mantle, the gills, and the palpi are heavily ciliated.)

❑   Notice that the foot is attached to the valves via anterior retractor muscles (dorsal to the labial palps) and posterior retractor muscles (dorsal to the posterior adductor muscle). What is the function of these muscles?

Squid

❑   Place the squid in your dissecting pan with the tentacles and head toward you and the funnel facing up. Note the mantle incision through which the latex was injected. Using medium scissors, continue this incision through the muscular mantle to the apex of the animal. About midway, you will find a small artery leading to the mantle; sever it close to the mantle. Be careful not to cut into the internal organs. Cut off the large flaps of mantle along the sides of the body. The **visceral mass** is now visible within the mantle cavity.

❑   The mantle is composed of a thick layer of circular muscles. The funnel has both circular and longitudinal muscles; the latter continue into the large prominent pair of **retractors** (not visible in Figure 7.7) which attach dorsally to the pen. Cut the funnel medially (along its midline) and note the liplike valve that regulates the flow of water through the funnel.

❑   Extending a short way into the funnel is the white **rectum**, with its opening between two small flaps, the **rectal papillae**. Dorsal to the rectum (below as you look at it) is the silvery **ink sac**. Do not puncture it. (If you do, rinse your specimen well.) The squid releases the ink or melanin pigment when it is being attacked, perhaps distracting or confusing the predator and interfering with its chemoreceptors for a short time.

❑   Determine the sex of your squid (refer to Figure 7.7). The large, whitish, paired **nidamental glands** cover the central portion of the visceral mass in a female. In a male, the slender, white **penis** will be seen on the left side, posterior to the rectum. You are responsible for knowing the reproductive organs and their functions for both sexes.
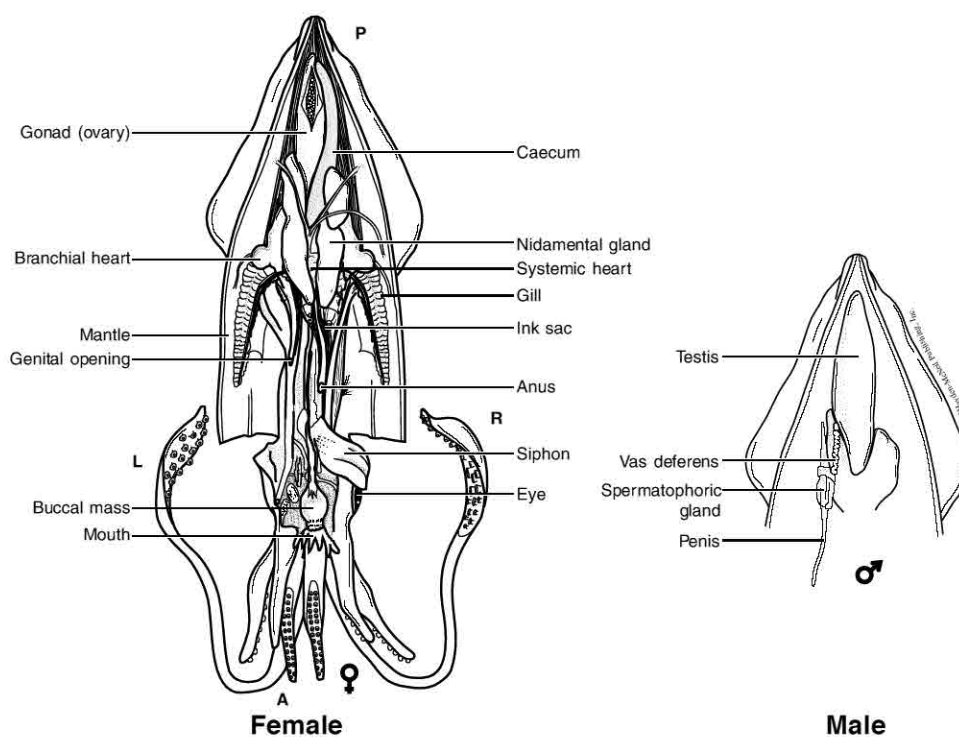
Figure 7.7. Internal anatomy of the squid (Mollusca, Cephalopoda), showing the female and male reproductive systems and other prominent organs.

BREAK 2 — Gross Internal Morphology

❑   Review what you have learned about both organisms.

❑   In both specimens, identify the major structures derived from the three basic molluscan body regions, the&

	Clam	*Squid*
head-foot:		
visceral mass:		
Mantle:		

❑   Review the mechanisms that move water through the clam and the squid. How are these mechanisms similar? How do they differ?

?   What structures of the clam perform a similar function to the openings around the collar and the funnel of the squid?

Organ Systems I (Respiratory, Circulatory, Excretory)

Clam

❑   Carefully remove the portion of the mantle covering the visceral mass and gills.

❑   Respiratory system. Observe the pair of folded **gills** on each side of the visceral mass. The gills are the clam's **gas exchange system**.

❑   Note that the two folds of each gill are marked by stripes running parallel from the dorsal to ventral border. These stripes are partitions that divide the interior of the gill into vertical water tubes. Make a cut across a gill at right angles to the partitions and note the cross-section of the water tubes on the cut surface.

❑   Observe the demonstration of a portion of gills on a microscope slide. Observe the ciliary action as streams of mucus flow along the gills trapping microscopic food particles and conducting them to the mouth. Thus the gill functions both in gas exchange and in nutrient procurement by filter feeding.

The respiratory system operates in the following manner: Water moves through the incurrent siphon and into the mantle chamber. Water then flows over the surfaces of the gills, which are lined with cilia and have numerous microscopic openings. The beating cilia create a current of water that flows through the minute openings into the vertical water tubes, which conduct the water dorsally to a canal and along it to the sub-branchial chamber. From this chamber, water exits from the animal via the excurrent siphon. A current of water thus circulates through the interior of the gills. Exchange of respiratory gases occurs through the walls of blood sinuses located in the partitions between the water tubes.

❑   Circulatory System. Carefully remove the thin wall of the pericardial cavity. A tube, the terminal part of the intestine, passes through the cavity. Wrapped around the central part of the intestine, is a spongy structure, the **ventricle** of the heart (Figure 7.8). The way to a clam's heart is really through the stomach! Look carefully at the ventricle to determine if it is still beating, perhaps very slowly.

❑   From the ventricle a very thin-walled, fan-shaped **auricle** extends downward on each side. The broadest part of each auricle is attached to the dorsal border of the adjacent gills from which the auricle receives aerated blood.

❑   From the anterior end of the ventricle, a vessel, the **anterior aorta**, leaves along the dorsal surface of the intestine. From the posterior end of the ventricle, a **ventral aorta** passes backward along the ventral surface of the intestine. These two aortae are difficult to see.

The circulatory system of the clam is the **open** type. Blood is pumped by the ventricle, leaves the heart via the aortae, and enters several arteries. Within the visceral mass, blood then leaves the arteries and moves through several large sinuses where it baths the organs directly before being collected by veins. Blood travels through sinuses in the excretory organs and then the gills before returning to the heart by the auricles.
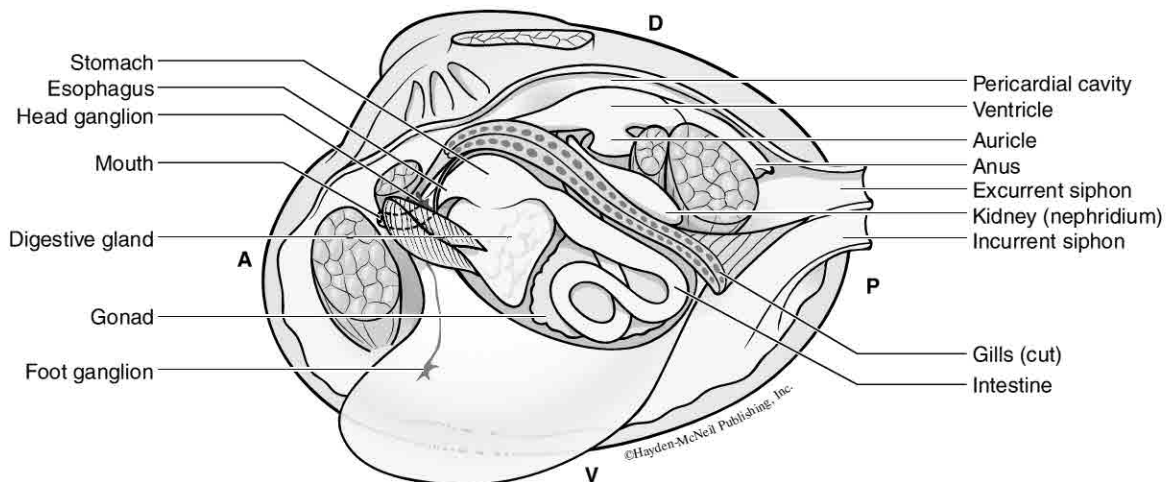
Figure 7.8. Internal anatomy (visceral mass) of a clam with left valve, mantle, gill, and part of the visceral mass removed (Mollusca, Bivalvia) (Helms & Helms 1989.)

❑ Excretory system: Expose the pericardial cavity fully to view the excretory system. Observe some brownish structures, which are a pair of **nephridia** in the floor of the cavity. The nephridia absorb nitrogenous waste from the blood and excrete it into the sub-branchial chamber near the excurrent siphon, where it leaves with the outflowing water.

Squid

❑ Female: (Refer to Figure 7.7.) The paired, white **nidamental glands**, which secrete the coverings for the egg capsules, are located over the kidney, rectum, ink gland, and heart region. Remove the nidamental glands by carefully prying them apart and lifting them off the other organs with a blunt probe. The **ovary** is a large, egg-filled organ that extends to the apex of the visceral mass. The **oviduct** with its flared opening lies parallel to the rectum. The oviduct begins as a small ciliated funnel near the ovary, loops anteriorly as the glandular, thicker-walled **oviducal gland** and terminates in the flared opening. Eggs are shed directly from the ovary into the coelomic cavity where they are fertilized. Fertilized eggs are then swept into the ciliated funnel of the oviduct, where they receive a gelatinous coat from the oviducal glands and an elastic membrane from the nidamental glands.

❑ Male: (Refer to Figure 7.7). After you have observed the respiratory system below, complete this section. You may remove the left gill, branchial heart, and posterior vena cava. The single large, white **testis** is located at the apex of the visceral mass, lying beside and slightly under the thinner-walled caecum. Sperm exit directly to the coelom by a slit at the anterior end of the testis. They are swept into the ciliated opening of the **vas deferens**, an opaque, white, tightly coiled tube, which leads into the thick-walled, large, coiled **spermatophoric gland**. The **penis** is the slender white tube lying to the left of, and slightly posterior to the rectum. Continue with the excretory system below.

During the breeding season, the male squid exhibits courtship behavior during which he removes spermatophores (packets of sperm) from the opening of the penis with a modified fourth arm and deposits them within the mantle cavity of the female. Following courtship and fertilization, the female attaches the gelatinous egg masses to a rock. The larvae pass through a rudimentary trochophore stage, and the young squid hatch within 2-3 weeks.

❑ Respiratory system: The large, feather-shaped **gills** lie along the sides of the mantle cavity. The inhalant streams of water pass over the gills and then converge to exit as a single exhalant stream through the funnel. The distal ends of the gills are attached to the mantle by a membrane. The blue latex-filled bulb at the base of each gill is the **branchial heart**, which pumps blood through the gill. Oxygenated blood returns to the systemic heart through the red latex-filled **branchial vein**, which runs along the outer edge of the gill. If your squid is a male, investigate the reproductive system now.

❑ Excretory system: Between the bases of the gills is a bulbous, blue latex-filled organ, the **kidney**. The kidney is indistinguishable from the precava of the circulatory system and closely associated with the branchial hearts at the base of the gills; contraction of the hearts creates a suction that draws fluid through the walls of the blood vessels into the kidney. The kidney also filters wastes from the coelomic fluid, which enters into a portion of the kidney through pores. The kidney ducts, or nephridiopores, excrete wastes into the anterior mantle cavity near the anus.

Squids have a double circulation plan. The branchial hearts pump deoxygenated blood into the gills, and the systemic heart pumps oxygenated blood through anterior and posterior aorta to the body. Anterior and posterior vena cavae collect deoxygenated blood and return it to the branchial hearts. Between arteries and veins, the blood travels through networks of capillaries in which the respiratory exchange of gases takes place. This closed circulatory system contrasts with the open system found in other mollusks. Refer to Figure 7.9 to help you understand the circulation of blood through the squid.

a.
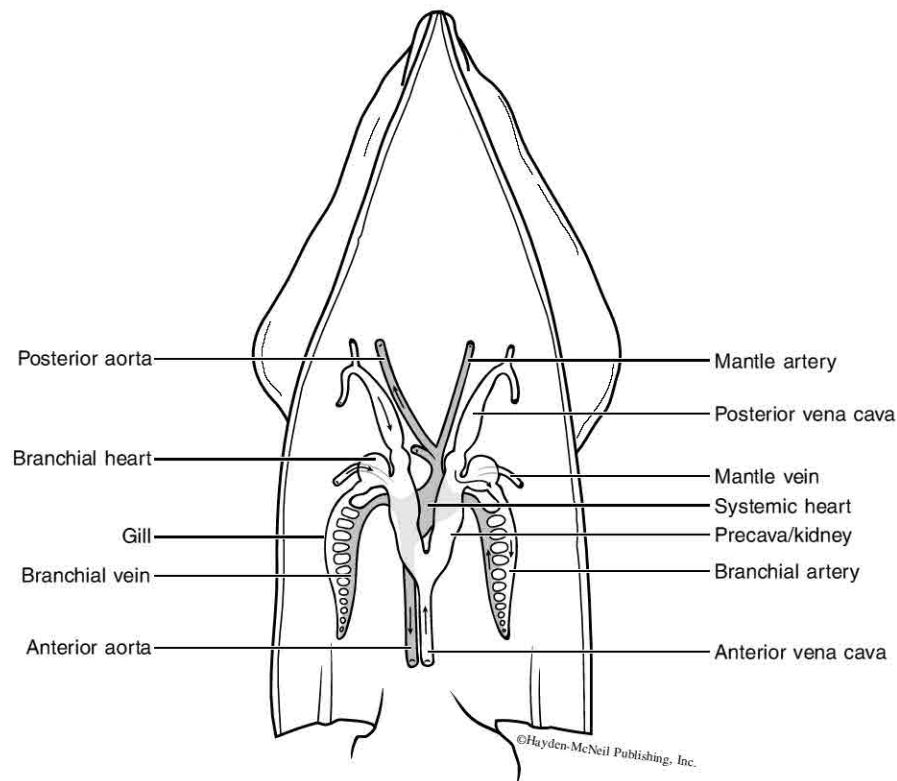
b.
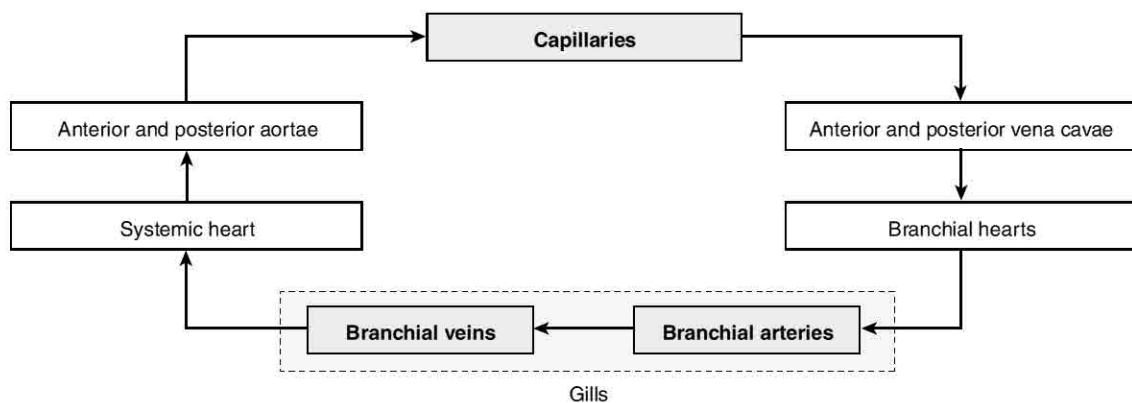
Figure 7.9. The double circulation system of a squid. a, clear vessels carry deoxygenated blood and are injected with blue latex in the preserved squid. Shaded vessels carry oxygenated blood and are injected with red latex. Arrows indicate the direction of blood flow. b, the schematic depiction of the double circulation. If dividing the circulation in the middle into halves, the left side of the system carries oxygenated blood and the right side is deoxygenated. The capillaries and gills (in shaded boxes) are the locations for gas exchange.

❑   Circulatory system: You have already located the branchial hearts at the base of the gills. These hearts receive venous blood from the large veins that drain the body. From the head, a single, large, blue latex-filled **anterior vena cava** proceeds medially to the kidneys. It divides into right and left **precavae**, which enter and pass through the renal sacs that surround the kidneys and then empty into the branchial hearts. Blood from the posterior part of the body passes through the large

right and left **posterior vena cavae**, the prominent, cone-shaped, blue latex-filled vessels. When the branchial heart contracts, it pumps blood to the gills through the **branchial artery**, a blue latex-filled vessel along the dorsal edge of the gill filaments.

❑   Gently scrape away the nephridial-precava tissue between the bases of the gills. The single, asymmetric systemic heart (filled with red latex) lies under this area. (The convoluted mass beneath the kidney on the right side is the pancreas. It is difficult to identify.) The two branchial veins, which you have already found in the gills, return oxygenated blood to the systemic heart. Two aortae leave the heart. The anterior aorta runs anteriorly parallel to the esophagus. The posterior aorta divides into three mantle arteries: the median one, which you saw as you opened the mantle cavity, and the paired lateral arteries, which supply the dorsal surface of the mantle cavity.

BREAK 3 — Organ Systems I

❑   Review what you have learned about both organisms.

❑   Consider the similarities and differences in the respiratory systems in these two mollusks. What aspect of their life history may have led to the differences you observe?

?   What are two similarities in the organization of the excretory organs in the squid and the clam?

1.

2.

❑   Compare and contrast the features of the circulatory system in the clam and the squid.

Clam	Squid

?   What features of the squid's circulatory system may allow it to lead a more "active" life than the clam?

Organ Systems II (Digestive, Reproductive, Nervous, Skeletal)

Clam

❑   Digestive system: Locate the mouth again. Beginning at the mouth, use a razor blade to make a sagittal section to carefully remove the left side of the visceral mass. The visceral mass is filled with the organs of digestion and reproduction held together by connective and muscle tissue. On the cut surface, the digestive tract appears as white tubes.

❑ The mouth leads into an enlarged cavity, the stomach (which you may not be able to see), surrounded by a pair of greenish digestive glands. From the stomach, the intestine makes several loops within the visceral mass and proceeds dorsally to emerge into the pericardial cavity. The posterior part of the intestine has already been seen as it passes through the pericardial chamber. Trace it now posteriorly and see if you can find the anal opening on the dorsal surface of the posterior adductor muscle, near the excurrent siphon.

Food particles that have become trapped in mucus on the surface of the gills and labial palps are conducted to the mouth by cilia. Cords of food-containing mucus are wound into the stomach by a crystalline style, composed of crystallized digestive enzymes. The style is located in a cilia-lined pouch within the stomach. The cilia cause the style to rotate. Enzymes released by the style begin the digestion of the food. Because the style dissolves quickly if the clam is forced to keep its valves closed for very long, it is seldom seen in captured specimens. Can you find any remnants of it?

❑ Reproductive system: Observe a pair of yellow-orange branched structures (either ovaries or testes) that are diffused throughout the visceral mass between the coils of the intestine in the section made above. Gametes are released into the sub-branchial chamber by ducts from the gonads. The sexes are separate in most species of bivalves.

❑ Nervous system: In the clams, as in most mollusks, cephalization is expressed as a chain of ganglia that surround the esophagus. In the clam the nervous system is considerably reduced compared to that of the other mollusks. We will not attempt to locate the ganglia.

Are you a lover of raw clams? Notice what you are eating the next time you have a clam and enjoy!

Squid

❑ Digestive system: (Refer to Figure 7.10). Remove the funnel and its retractor muscles. Make a medial lengthwise incision to separate the ventral pair of arms and expose the **buccal mass**, a muscular oval organ that bears two horny **beaks** used for ripping prey. Flatten the arms and tentacles to expose and study this area. Open the buccal mass and observe the toothed **radula**. Posterior to the buccal mass are a pair of salivary glands, which release their poisonous secretions into the buccal cavity.

❑ The **esophagus**, a thin-walled narrow tube, leads out of the buccal mass, passes dorsally through the **liver**, and joins the thick-walled **stomach** near the middle of the visceral mass. The liver is a large, cone-shaped whitish organ with its broad base near the collar. (It lies between the large retractor muscles of the funnel and may be confused with them.) The stomach leads into the **caecum**, a long, thin-walled sac, which may extend to the apex of the mantle cavity. The ducts of the liver and pancreas empty into the caecum, where digestion and absorption take place. The short intestine leads from the stomach, runs forward and becomes the **rectum**. The ink sac is a pouch off the intestine.

❑ "Skeletal" System: The chitinous **pen**, located in the dorsal wall of the mantle and extending from the edge of the collar to the apex, is the internal shell of this mollusk. Locate and remove it. A number of internal **cartilages** support muscles and form interlocking surfaces around the collar. A large piece of cartilage also encloses and protects the brain.
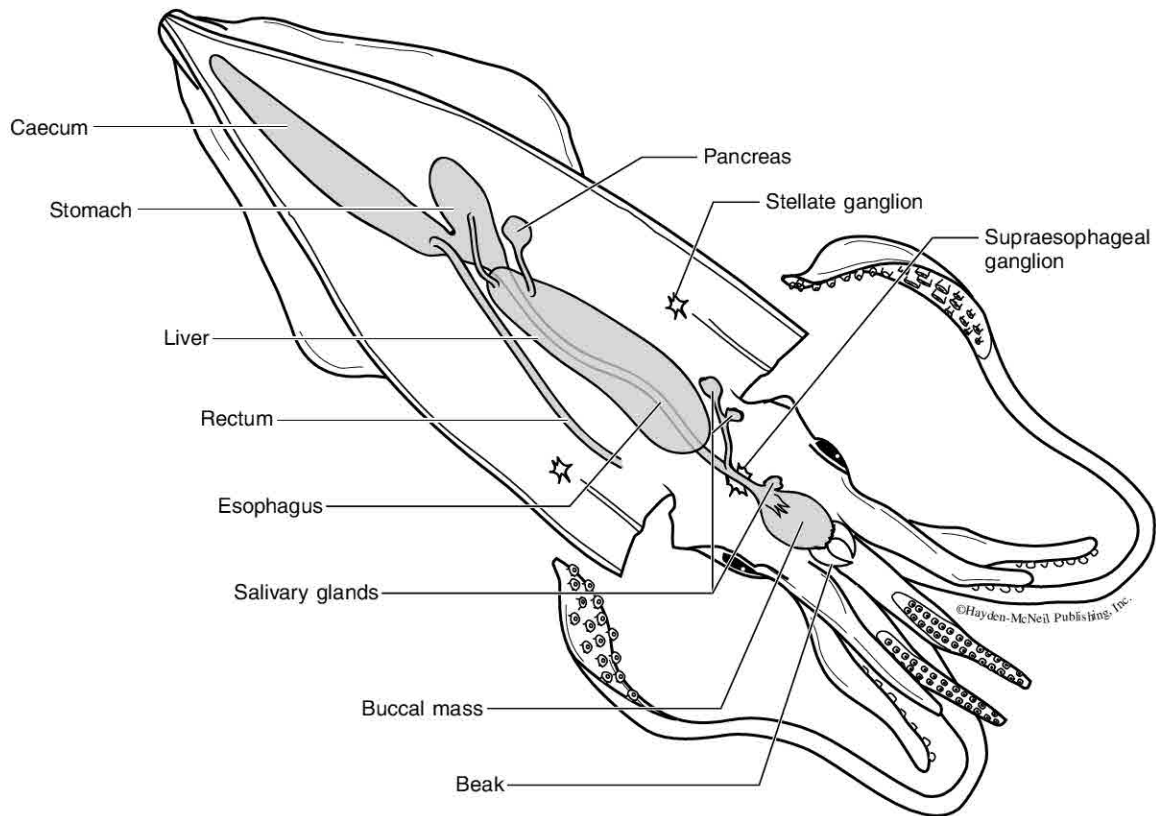
Figure 7.10. The digestive system of the squid (Mollusca, Cephalopoda). (Funnel has been removed.)

❑   Nervous System: The brain of the squid consists of several pairs of enlarged, fused ganglia, located above the esophagus posterior to the buccal mass. Cut a median sagittal section through the head with a razor blade and look for the **supraesophageal ganglion** (a single rounded mass above the esophagus) and other ganglia below the esophagus. A pair of large mantle nerves run from these ganglia to the large **stellate ganglia**, located on the inner dorsal surface of the mantle just anterior to each gill. These motor centers are the points of synapse in the giant fiber system, which rapidly relays messages to the mantle muscles to assure fast locomotion. The squid's giant axons are a favorite material for neurophysiological study.

BREAK 4 — Organ Systems II

❑   Review what you have learned about both organisms.

?   What features of the digestive system of the clam and squid seem to be related to their mode of nutrition?

? How might you explain the observation that the clam has a relatively long intestine while the squid's is quite short?

? What functions in the clam and the squid depend on movement of water through the organism?

Clam	*Squid*

CONCLUDING ACTIVITIES AND DISCUSSION

❑ Before you dispose of your squid and clam, your lab instructor will expect you to demonstrate that you have correctly identified all important structures by providing him/her with a "tour" of your specimens.

❑ Review your understanding of the functions of the various systems for Cnidaria, Platyhelminthes, Nematoda, Rotifer and Mollusks by completing Table 7.1 (p. 160).

DAY 2

PROTOSTOMIA: PHYLUM ANNELIDA (Lophotrochozoan Clade)

The phylum Annelida, the segmented worms, is divided into three classes: Polychaeta (segmented marine worms), Oligochaeta (earthworms), and Hirudinea (leeches). The obvious external **segmentation** of these worms is usually continued internally as a series of compartments separated by partitions called **septa**. These fluid-filled segments create a **hydrostatic skeleton** against which the longitudinal and circular body muscles can contract and produce movement. Annelids have a well-developed coelom separating the muscular gut from the body wall, a <u>closed</u> circulatory system, well-developed reproductive and excretory systems, and an anteriorly concentrated nervous system. **Nephridia**, units of the excretory system, and ganglia on the **ventral nerve cord** often occur segmentally throughout the body. We will look at representatives of the classes Oligochaeta and Hirudinea.

Class Oligochaeta - the earthworms

The earthworms show specializations for a burrowing existence. Head appendages and anteriorly-placed sense organs are absent. Each body segment has only four pairs of setae (bristles) and no parapodia (paired, segmentally arranged, lateral, flaplike protuberances found in polychaete worms). Gas exchange takes place through the skin, which is kept moist by the secretion of coelomic fluid and mucus.

Earthworms are **hermaphroditic** (they possess both male and female reproductive structures). However, the earthworms use cross-fertilization. Mating worms face in opposite directions so the clitellum of each lines up with the other's spermathecal openings (sperm ducts). Sperm, produced by the testes, mature in three pairs of **seminal vesicles** (irregularly shaped), and are discharged via the sperm funnel into the sperm ducts. Eggs, produced by the ovaries, are released into the coelom where they are collected by funnels that lead to the female genital pores on segment 14. During copulation, the clitellum of one worm attaches to the segments containing the seminal receptacles of the opposite worm. Each worm secretes a slime tube about itself and the genital setae hold the worms firmly together. Masses of sperm traverse the seminal grooves to enter the seminal receptacles of the other worm. Following copulation, the worms separate and the clitellum secretes a thick, chitinous-covered mucus band that slips forward along the worm, picking up eggs from the oviducts and stored sperm from the seminal receptacles. The mucus band, complete with eggs and sperm, is then deposited as a cocoon in which the worm larvae develop.

Dissection of the Earthworm (*Lumbricus* sp.)

You will be working in groups of two students for this dissection. Note: you will need to carefully use the binocular, stereoscopic "dissection" microscope to be able to see clearly all structures being considered. Obtain an instrument from the microscope cabinet and set it up for use now.

❑   Obtain a live earthworm. Observe its movement first on wet paper toweling and then on the smooth lab bench surface.

?   How is forward movement accomplished? On which surface does it move best? How can you explain this?

❑   Note the waves of body contraction and extension as the worm moves forward and backward. Also, feel the setae with which the worm anchors itself to the substrate.

?   The body wall of the earthworm is composed of an outer layer of circular muscles and an inner band of longitudinal muscles. Describe how the alternate contraction of these muscles in conjunction with the internal, fluid-filled body segments acts to create the movement that you observe?

❑   Return the live worm to its container and obtain a freshly-killed worm for the following dissection. Note: these worms were euthanized by placing them in 35% ethanol for several minutes.

❑   Identify the dorsal side by the presence of a dark, pulsating line, the **dorsal blood vessel**. Locate the following external features on the earthworm: (See photographs)

1.   **Mouth** with the overhanging lip called the **prostomium.**

2.   **Clitellum** - enlarged band of segments that secrete mucus during reproduction.

3.   **Setae** - bristles occurring in four pairs, two laterally and two ventrally on each segment.

4.   **Sperm duct pores** - openings (somewhat swollen) on ventral surface of 15th segment.

5.   A pair of **seminal** or **sperm grooves** on the ventral surface extending from segment 16 to the clitellum.

6. **Genital setae** - a pair of swollen areas on segment 26 with setae that help bind worms, facing in opposite directions, together during mating

❑   Place the worm dorsal side up toward the edge of a dissection pan (so that you can perform this dissection under the stereoscopic microscope). Secure the worm with one pin through the third segment and another about 5 cm behind the clitellum. (Remember that the clitellum is closer to the anterior than the posterior end.)

❑   With the tips of your fine scissors pointing up, make a mid-dorsal cut starting just in front of the clitellum and proceeding anteriorly. Using forceps and a dissecting needle, free the body wall from the **septa**, the membranes that form the partitions between segments. Pin the body wall flat.

❑   Submerge your worm in Ringer's solution. Use Figure 7.11 as a guide for your dissection.

❑   Circulatory system: Locate the **dorsal blood vessel**, partly embedded in the top of the intestine. Observe it under the dissecting microscope. Is it pumping? Can you tell which direction the blood is flowing? In segments 7 through 11, most of the blood passes from the dorsal blood vessel through the five **aortic arches** (or pseudohearts) to the **ventral blood vessel**. Can you see the contraction of the aortic arches?

❑   Digestive system: The **mouth** leads into a short tube called the **buccal cavity**. On the dorsal surface of this tube, where it connects with the large muscular **pharynx**, is the small white bilobed brain or **suprapharyngeal ganglia**. The thick pharynx, the principal organ of ingestion, narrows to form the **esophagus**, which runs through the region of the aortic arches.

❑   Two pairs of whitish **calciferous glands** are located on either side of the last aortic arch. These esophageal outgrowths are excretory organs and probably function in regulating the level of calcium ions in the blood and in maintaining a constant pH. Gently probe the next two compartments of the digestive tract—the **crop** and the **gizzard**.

?   What can you conclude about the functions of the crop and gizzard, based on your probing? What structural features support your conclusions?

❑   The remainder of the digestive system is the thin brown intestine, which extends to the anus. Slice open a short segment of the intestine laterally, and observe the mid-dorsal thickening which serves to increase the surface area of the gut. The yellowish covering on the intestines is made up of **chloragogue cells**, which function in glycogen synthesis, deamination of proteins, and formation of ammonia and urea.

?   To what vertebrate organ are chloragogue cells analogous? _____

❑   Reproductive system: Oligochaetes are hermaphroditic, and therefore have gonads for both sexes, although cross-fertilization is the rule. The large, white, trilobed **seminal vesicles** partly cover the aortic arches in segments 9-13. Sperm produced in the testes (which are associated closely with the seminal vesicles and cannot be easily seen) are stored in the seminal vesicles and then released during copulation through the sperm ducts, seen externally on segment 15. Two small **ovaries** hang from the anterior septum in segment 13. The **ovarian funnel**, also in segment 13, leads into the **oviduct** in segment 14. These female structures are usually hard to find. The sperm received during copulation are temporarily stored in the **seminal receptacles**, whitish round sacs seen in segments 9 and 10.
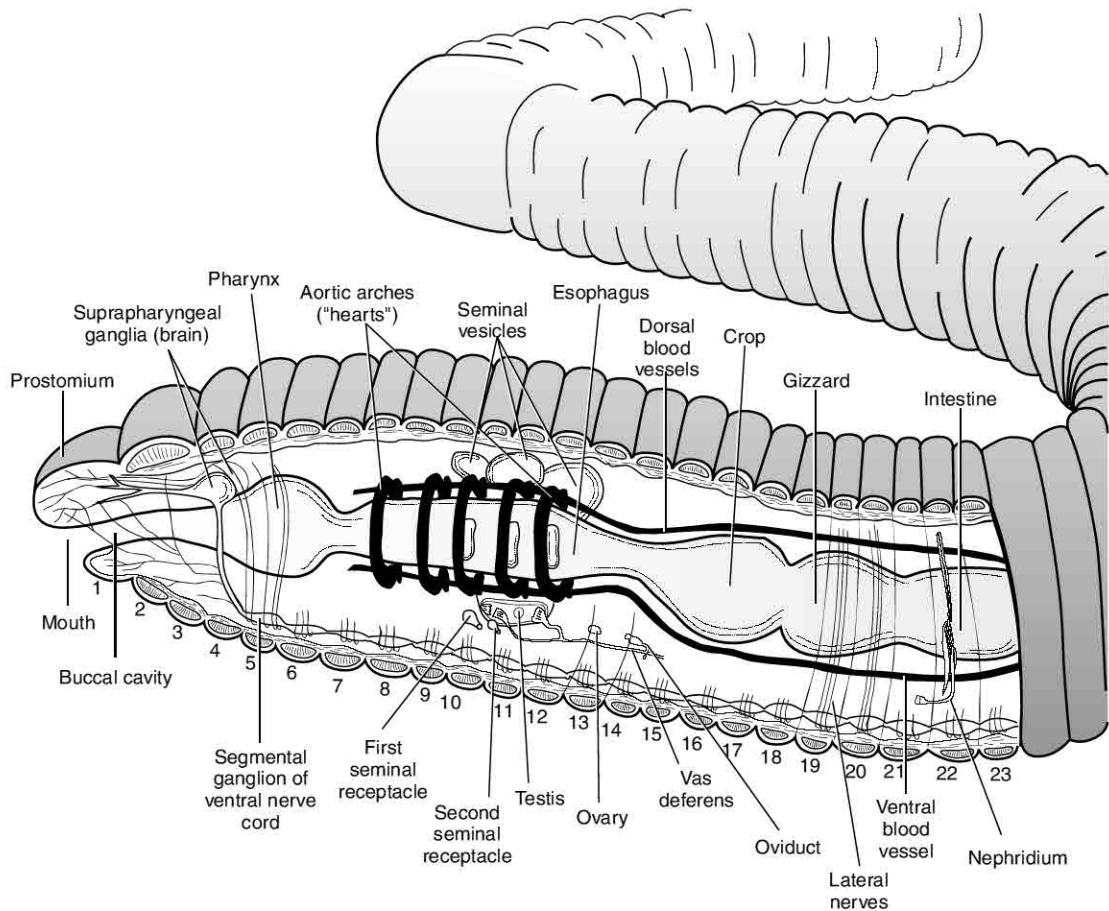
Figure 7.11. Dorsal and ventral view of the internal anatomy of an earthworm (Annelida, Oligochaeta).

❑ Excretory system: In the earthworm, every segment except the first three and last one contains a pair of **nephridia**. To see a nephridium, carefully focus at about 15X with the dissecting microscope on the septa to the side of the digestive tube. A ciliated funnel, the **nephrostome**, is situated posteriorly in each segment and leads, by way of a duct, through the intersegmental septum into a coiled **tubule** surrounded by blood capillaries located in the next posterior segment.

The tubule leads into a **bladder** that empties to the exterior through a **nephridiopore**, an opening near the ventral pair of setae. The cilia of the funnel and tubule beat and draw coelomic fluid into them. Wastes and other materials are exchanged with the blood in the capillaries.

❑ Nervous system: Relocate the **suprapharyngeal ganglia**, or "brain," on the dorsal anterior edge of the pharynx. Sever the esophagus from the posterior end of the pharynx and move the digestive tract and accompanying glands to the side. Loosen and lift the pharynx and observe the **subpharyngeal ganglia**. The **ventral nerve cord**, a white cord in the mid-ventral line, proceeds posteriorly. Note the enlarged **segmental ganglion** on the cord in the center of each segment. Observe the lateral branches from the ventral nerve cord and look for the nerves running from the brain to the mouth region.

❑ Examine the slide of the transverse-section of an earthworm under low power on your compound microscope and look for the structures that you located in your dissection. Label these structures in Figure 7.12.
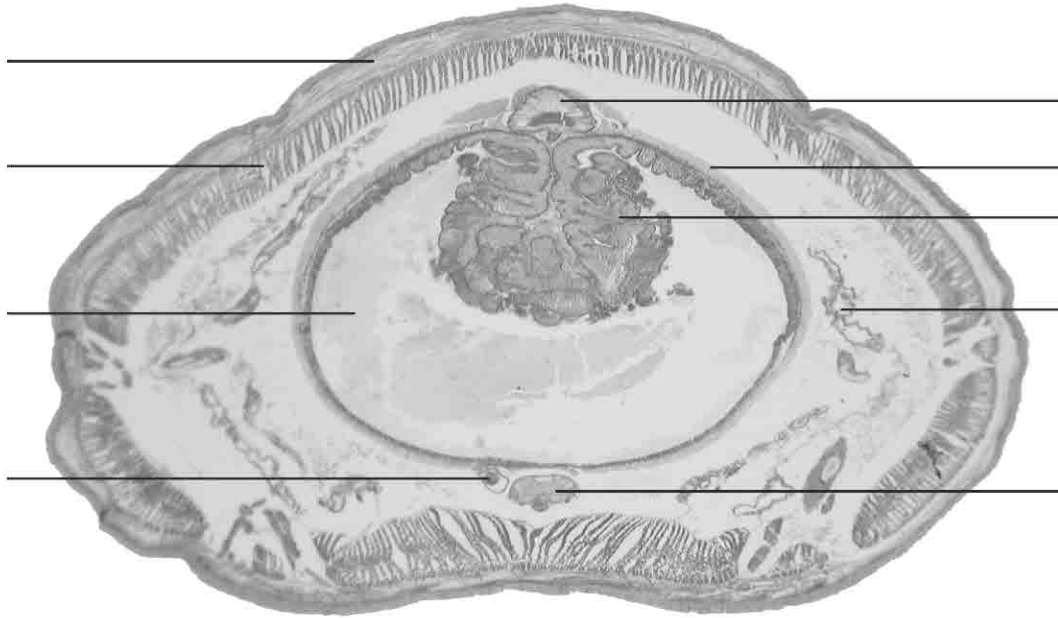
Figure 7.12. Photomicrograph of the transverse-section of an earthworm (Annelida, Oligochaeta). Label the indicated structures.

❑ On the earthworm slide, note the central **intestine** with its dorsal fold, the **typhlosole**, and the **coelom** between the gut and the body wall. (Hint: the solid circular structure in the center of the cross section is the typhlosole, not the intestine. The intestine is the hollow chamber around the typhlosole.) In the coelom may be seen traces of nephridia, blood vessels, and septa. Identify the outer **circular muscle** layer of the body wall and the inner feathery layer of **longitudinal muscle** interrupted in four places for the insertion of the setae. Find the **dorsal blood vessel** lying above the intestine, the **ventral blood vessel** below the intestine attached by the ventral mesentery, and the **ventral nerve cord** lying on top of the longitudinal muscle. Note the thickened covering of **chloragogue cells** on the dorsal surface of the intestine.

❑ Review your understanding of earthworm anatomy and the functioning of the circulatory, reproductive, respiratory, and locomotory systems in this animal and complete the Annelida section of Table 7.1 (page 160).

Class Hirudinea - the leeches

❑   Observe the demonstration of live leeches. Pay particular attention to the movements they make
       while swimming and creeping (looping).
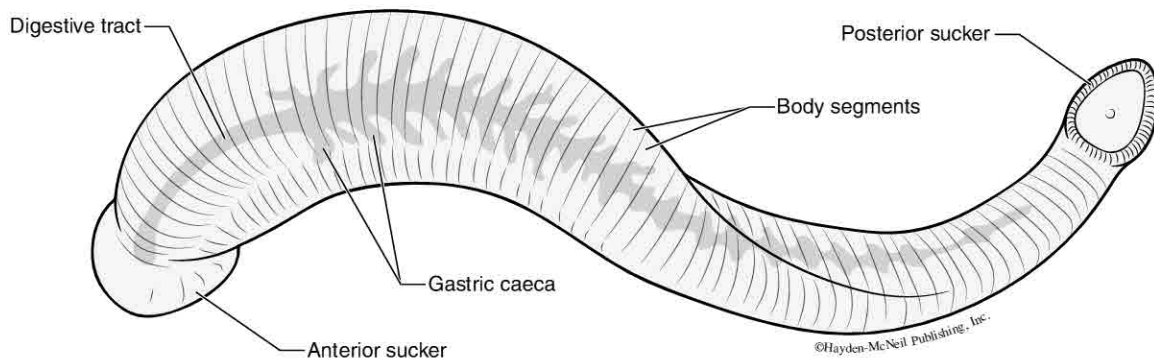
Figure 7.13. Simplified diagram of a leech showing gastric caeca. (Annelida, Hirudinea).

        Leeches are predominantly a freshwater group found in lakes, ponds, marshes and slow moving
streams. Their dorso-ventrally flattened bodies bear anterior and posterior suckers for attaching to their
prey and for locomotion (Figure 7.13). They do not burrow or crawl as do other annelids but move along
with a looping movement much as does an inch worm or hydra. With each step they alternately attach
and release the anterior and posterior suckers. This type of locomotion does not depend on a spacious
hydrostatic skeleton and the body of leeches is nearly solid; the coelom is largely filled with connective
tissue. Much of the internal segmentation common in other classes of annelids is absent in these animals.
Blood sucking leeches have numerous extension from their gut called **caeca**, which increase surface area
for digestion and are also used to store blood.

PROTOSTOMIA: PHYLUM ARTHROPODA (Ecdysozoan Clade)

        The Arthropoda (*arthros*, joint; *podos*, foot; Gk.) is the largest animal phylum, comprising over 80%
of all animal species. This phylum is home to the insects (the most abundant group), crustaceans
(crayfish, shrimp, crabs, daphnia), spiders, centipedes, and millipedes. The major characteristics of this
phylum are a **segmented body**, a **chitinous exoskeleton**, and **jointed appendages**.

        The bodies of most arthropods are divided into three major regions: the **head**, **thorax**, and
abdomen. The exoskeleton functions for support and protection as well as locomotion. To grow, an
arthropod must molt, shedding its exoskeleton and secreting a new, larger one. (This process is called
ecdysis, the basis for the clade name Ecdysozoa.) The appendages have been highly modified for a large
variety of functions. A well-developed musculature is attached to the inside of the exoskeleton and moves
the jointed appendages. The nervous system consists of a ventral nerve cord with segmental ganglia and
shows considerable cephalization. The circulatory system is open, the coelom is reduced, and the
respiratory organs are usually gills or trachea.

        There appear to be four main evolutionary lineages of arthropods: the trilobites (represented only as
fossils of extinct marine members), the chelicerates, the uniramians, and the crustaceans. Chelicerates
(horseshoe crabs and arachnids--spiders, ticks, scorpions) have fanglike or pincerlike mouthparts called
chelicerae but lack antennae and mandibles. The uniramians and crustaceans have **mandibles** instead
of chelicerae as their first pair of modified mouthparts. Mandibles function in biting and chewing (although

in some species they are secondarily modified for piercing and sucking). Uniramians (centipedes, millipedes, and insects) possess one pair of **antennae** as sensory organs and unbranched (uniramous) appendages; crustaceans have two pairs of antennae and branched appendages. Most members of these two groups have additional mouthparts called **maxillae** and a pair of **compound eyes**. Arthropod phylogenetics continue to be reinterpreted based on molecular data and cladistic analysis.

Subphylum Crustacea - the lobsters, crabs, and their relatives

This class includes a diversity of animals ranging from the familiar crayfish, shrimp, and crabs to *Daphnia*, sowbugs, and barnacles. Crustaceans characteristically have two pairs of antennae, a set of mandibles, and two pairs of maxillae. The rest of the appendages and even the sections of the body vary a great deal within this class. The wide variety of modifications to the jointed appendages of crustaceans are associated not only with feeding and swimming, but also with reproduction, respiration, and burrowing. The majority of the representatives are marine, but there are many freshwater species (e.g., crayfish and small crustaceans such as *Daphnia* and copepods, Figure 7.14) and a few terrestrial species, such as the sowbugs you may have studied during the orientation behavior lab in the first semester. Many tiny freshwater and marine crustaceans occupy an important position as zooplankton in aquatic food chains. You will examine the zooplankton typical of local ponds during a lab on freshwater ecology next week.
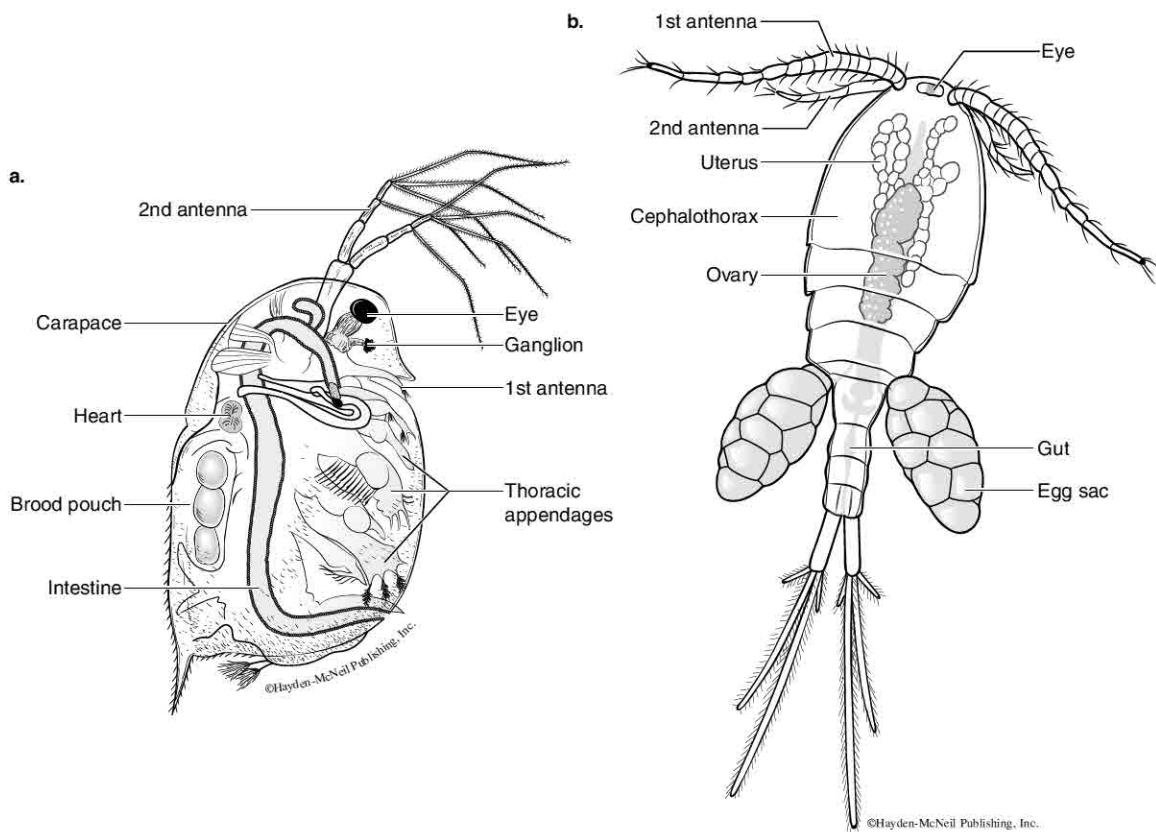
Figure 7.14. A. Diagram of *Daphnia* (Sherman and Sherman 1976.) Notice the dense setae hairs on the thoracic appendages. B. Diagram of a female copepod (Barnes 1974). Note the prominent egg sacs.

The crayfish, shrimp, and crabs are examples of the order Decapoda. The decapod body is divided into two main sections, a **cephalothorax** (covered by a portion of the exoskeleton called the **carapace**) and an **abdomen**. The first pair of the five pairs of thoracic appendages (hence the name *deca* -ten; *poda* -foot) is usually modified into pincers which function in capture of prey or defense.

Life History of the Crayfish, *Procambarus* sp.

Most North American crayfish species are freshwater dwellers and can be found in the shallow waters of streams and ponds. They dig burrows in or near water and remain hidden during the day. Crayfish are scavengers on both living and decaying plant and animal material. Crayfish are preyed upon by a variety of animals, including fish, wading birds, raccoons, and otters. In certain parts of the world crayfish are regularly collected and eaten by humans. The flavor of the tail meat is similar to that of their large relative the lobster. Other life history information will be presented in the sections describing the dissection.

Dissection of the Crayfish

Each group of three students should obtain a freshly euthanized crayfish from the lab instructor. Use of the stereoscopic, binocular microscope is essential in this dissection.

❑   Observe several live crayfish in a pan of water.

    1.   Describe the typical forms of locomotion you observe. How are the appendages used to walk? To swim?

    2.   Describe any defensive behavior your gentle probing elicits.

    3.   Can you make any observations of a resting individual that suggest how crayfish maintain a water current across their gills?

❑   Place the dead crayfish dorsal side up in a dissecting pan.

External Features: Refer to Figure 7.15 while identifying the external features of the crayfish.

❑   Note the general body plan of the crayfish. It is divided into a **cephalothorax** (anterior half) and an **abdomen** (posterior half). The dorsal portion of the cephalothoracic exoskeleton of decapods is heavily mineralized (with calcium and phosphate salts) and is called the **carapace.** The **cervical groove** which represents the nearly complete fusion of the head and thorax is a lateral seam (suture) across the carapace.

❑   The head of the crayfish ends anteriorly in a protective hood, the **rostrum**. On either side of the rostrum is a **compound eye,** situated on a stalk that allows the eye to move.

❑   Crustaceans have two pairs of **antennae**, used primarily as sensory appendages. Compare the structure of the two types of antennae. At the base of the long antennae, locate the small pores (**renal pores**) that function in the excretion of nitrogenous waste and excess water discharged from the osmoregulatory organs called the green glands (you will locate these glands later).
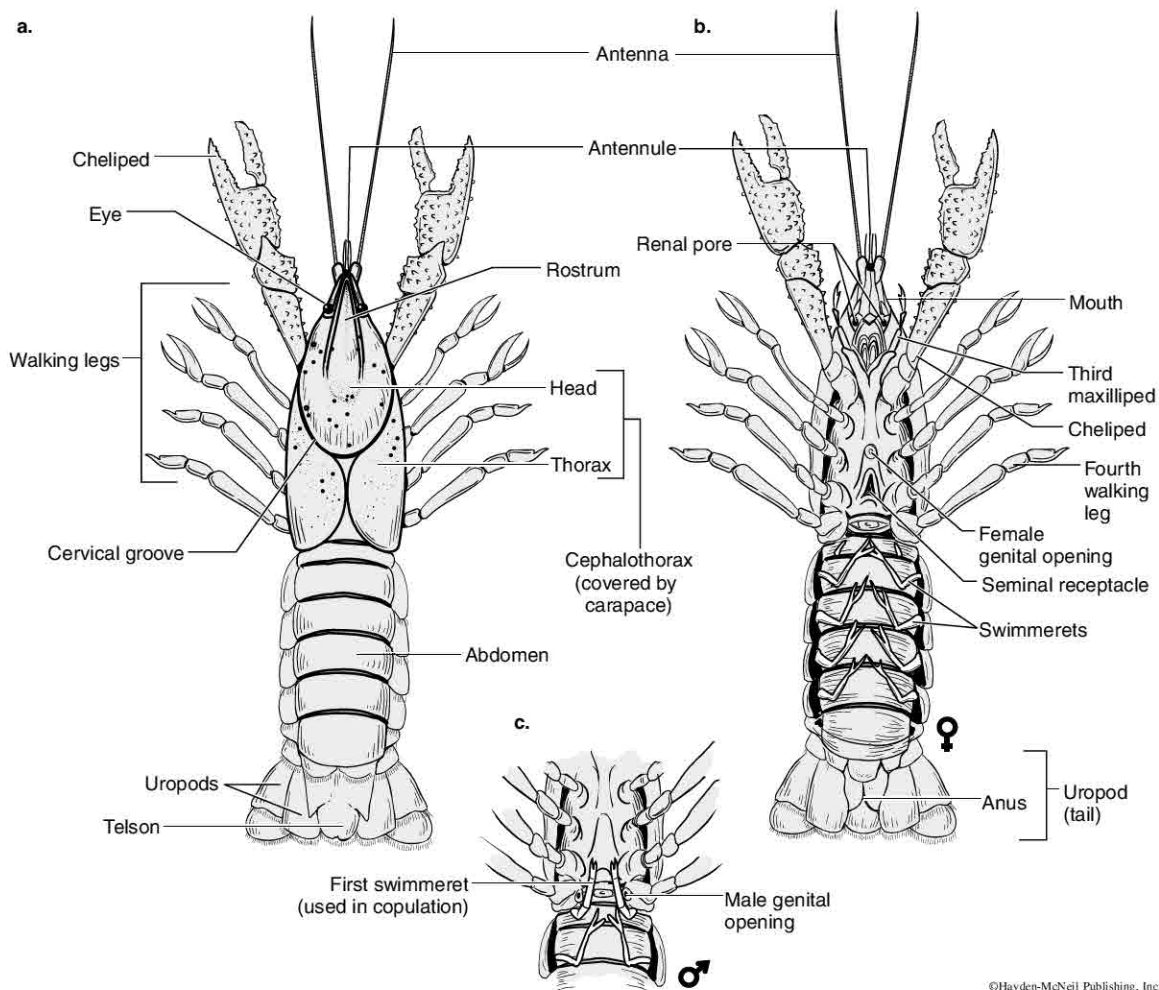
Figure 7.15. The external anatomy of a crayfish (Arthropoda, Crustacea): (a) Dorsal view, (b) Ventral view of a female, (c) Ventral view of a male (Helms & Helms 1989).

❑ Appendages: Lay the animal ventral surface up. In addition to antennae, crayfish have several other types of appendages.

(1) *Mouthpart appendages*: Surrounding the mouth are the heavy **mandibles** (jaws) that grind food, three pairs of large **maxillipeds,** and two pairs of **maxilla** that handle food and carry sensory organs for taste and touch. You will identify these structures shortly.

(2) *Walking appendages*: Five pairs are associated mainly with crawling and digging: the large appendages of the cephalothorax are the walking legs. The first pair of legs are called **chelipeds** because they bear pincers, called chelae, which are used in grasping and tearing food as well as in defense.

(3) *Abdominal appendages*: The most posterior six pairs of appendages, called **uropods** and **swimmerets**, are used mainly in swimming. Contraction of the ventral abdominal muscles draws the **telson** (the posterior projection of the last body segment) and uropods (shown in Figure 7.15) under the body, allowing the crayfish to swim rapidly backwards.

(4) *Reproductive appendages*: If your specimen is a male, the swimmerets on the first abdominal appendages have an odd spatulate shape and will be pressed closely together and pointing forward. They serve as sperm transfer organs (gonopods), receiving sperm from openings at the base of the last pair of walking legs. If your specimen is a female, these swimmerets are small. Between the bases of the third and fourth pairs of walking legs, the female has an exoskeleton swelling, the **seminal receptacle**, where sperm from the male are deposited during mating. Locate the seminal receptacle and the female genital openings at the base of the second pair of walking legs. Note: Be sure you observe both males and females in the lab.

During mating, the male grasps the female, inverts her, and holds her motionless. The two modified swimmerets are appressed against the seminal receptacle of the female, and sperm packaged in mucus are transferred into the seminal receptacle where they are stored. Days or weeks later, the female lies upside down and flexes her abdomen. The eggs are extruded from the oviducts and sperm from the seminal receptacle. Fertilization takes place externally and the fertilized eggs become attached to the swimmerets of the female. The eggs remain attached to the female for weeks, aerated by the movement of her swimmerets. A female in this condition with the eggs attached is said to be "in berry."

❑   Respiratory system: The **gills** are contained in the **branchial chambers**, located laterally under the carapace. Use scissors to lift and cut away one side of the carapace from the posterior half of the cephalothorax to the cervical groove (see Fig. 7.15 a). (Note: be careful not to damage the underlying gills.) Gradually clear away the material to reveal the feathery gills. Water is drawn in through an opening at the posterior end of the branchial chamber, passes over the gills, and exits anteriorly as a result of the action of a mouthpart appendage called the **gill bailer** (seen shortly). Remove a walking leg and notice that the gills are attached to each leg. What advantage does this give?

❑   Mouthparts: Observe the six pairs of appendages concerned with the handling of food. (Note: you are only responsible for being able to identify two of the six appendage types, whose names are shown in bold type below.) Beginning with the appendage just anterior to the cheliped, remove each appendage in order, working anteriorly. Arrange the mouthparts on a paper towel in the order they were removed. To remove, grasp the base of the appendage with forceps and gently pull it out. The first appendage anterior to the cheliped is the largest and most leg-like mouthpart appendage, the 3rd maxilliped; it consists of a number of projections. Notice the feathery gills which are attached to it. The next appendage anteriorly is the 2nd maxilliped; it too consists of a number of projections and has gills attached. The next appendage is the first maxilliped; it is smaller and lacks a gill. The next appendage is smaller yet; it is the 2nd maxilla or **gill bailer**. Its movement causes water to move across the gills. The 1st maxilla is a smaller, leaflike structure. The anterior-most appendage is the **mandible** which can be identified by its broad, smooth surface, inner sawlike edges, and the blunt fingerlike process attached at the anterior end. The heavy mandibles grind and crush food.

Why are you asked to locate all six mouthparts if you are required to know only two? The mouthparts are important from an evolutionary standpoint. During the course of arthropod evolution, the number of segments has been reduced, the segments have been grouped into distinct body regions, the number of legs has been reduced, and the legs on some segments have been modified for functions other than locomotion. These trends are apparent in the crayfish. Note that each segment bears a pair of appendages, the more ancestral condition, but that some legs have been modified for food handling rather than locomotion.
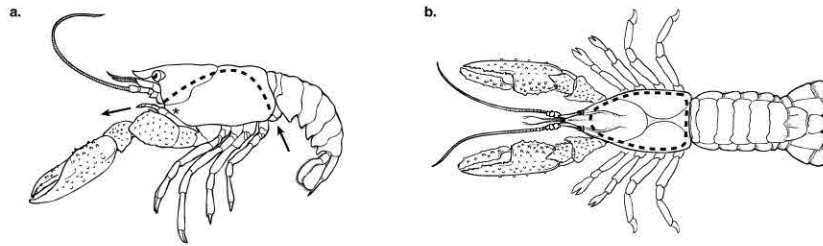
Figure 7.16. Cuts to be made on crayfish carapace: (a) side view: dotted line shows cut to view gill. Asterisk indicates position of gill bailer, arrows indicate direction of respiratory current; (b) dorsal view: dotted line shows cut to expose the heart prior to internal dissection.

Internal anatomy: Refer to Figures 7.15, 7.16 & 7.17 while identifying the internal features of the crayfish.

❑ Now you will examine the internal organs. Pin your specimen securely, dorsal side up, in a wax-lined dissecting dish. Arrange the specimen so that it may later be viewed under the dissecting microscope without removing any pins. With scissors, carefully remove the dorsal carapace (Figure 7.16 b). Work forward and backward from where you earlier removed the lateral carapace removing small pieces of exoskeleton as you go. Keep the instrument points up; tissues beneath the carapace are soft and easily destroyed. Use a blunt probe to carefully separate the carapace from the underlying hypodermis, the tissue that secretes the carapace. Completely remove the carapace. Keep the specimen moist with tap water. Use forceps and scissors to carefully remove the red-speckled membrane in the dorsal wall of the **pericardial sac.**

❑ Circulatory system: The heart is a delicate sac located in the posterior third of the cephalothorax. It is suspended within the pericardial sac by three pairs of ligaments. Blood (hemolymph) enters the heart from the body cavity through **ostia** (small holes in the heart wall) during the heart's relaxation phase. When the heart contracts small flaps of tissue seal the ostia from inside the heart, and blood is forced out into arteries. Several arteries carry hemolymph away from the heart and release it directly onto the tissues. From the tissues, hemolymph percolates back through the gills to the heart. These vessels are difficult to find unless the circulatory system has been injected with colored latex.

Hemocyanin, the respiratory pigment in crustaceans, is structurally similar to hemoglobin, but contains copper not iron. It is not carried in blood cells but dissolved in the plasma and gives crustacean hemolymph a bluish tint.

❑ Reproductive system: Remove the heart and the thick wall of the pericardial sac below the heart. The reproductive organs of both sexes are located underneath the heart. Both the **ovaries** and **testes** (Figure 7.17 a & b, respectively) are approximately Y-shaped, with the upper branches of the Y extending anteriorly and the lower branches running posteriorly some distance towards the abdomen or into it. The **ovaries** are brownish-orange in color and filled with eggs. Each of the two fragile oviducts opens on the base of the second walking legs. Sperm ducts carry male gametes to openings on the bases of the last pair of walking legs. The reproductive structures may be difficult to locate unless the animal is in breeding condition.

a.

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b.

©Hayden-McNeil Publishing, Inc.
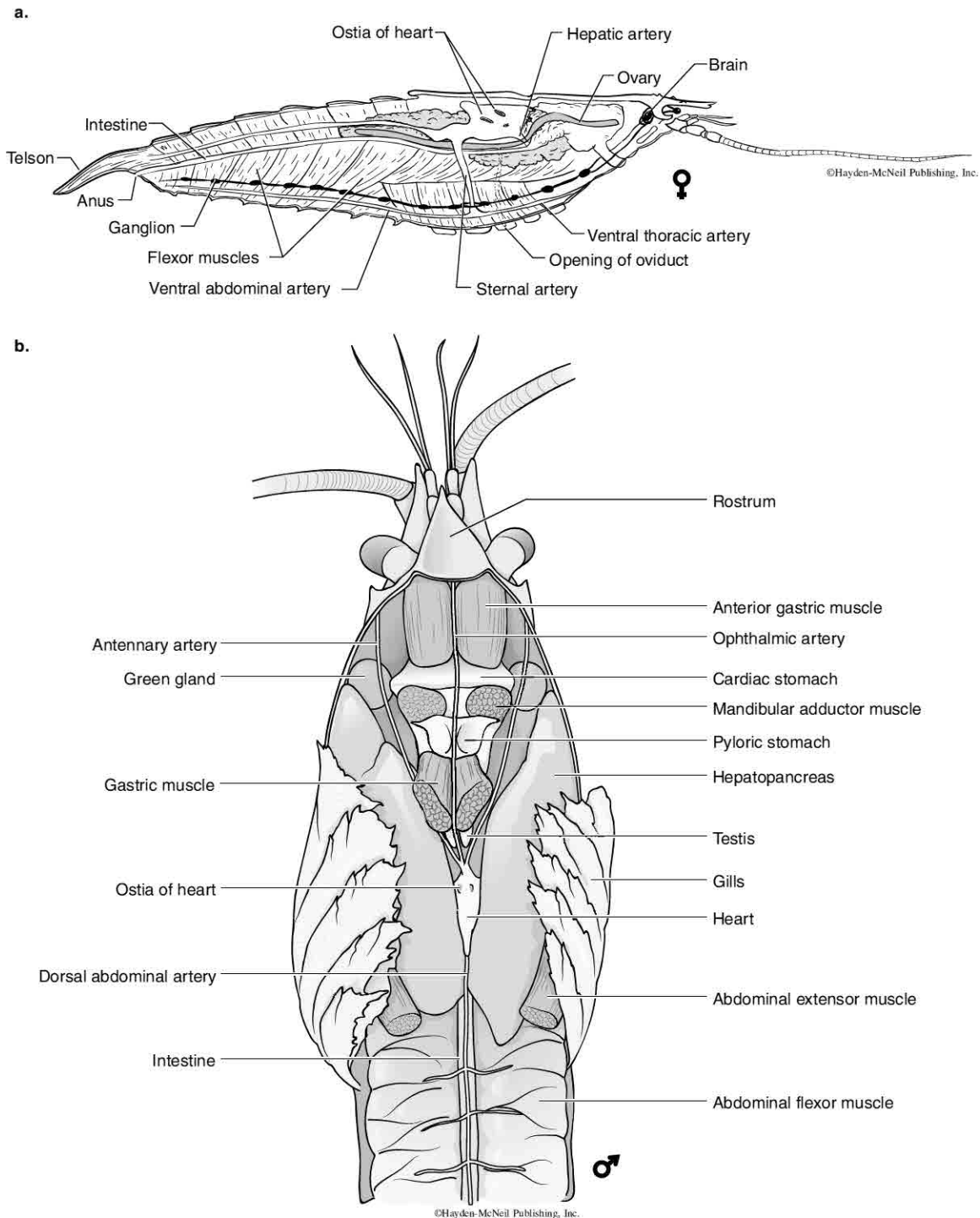
Figure 7.17. The internal anatomy of a crayfish (Arthropoda, Crustacea) (a) lateral view (female) and (b) dorsal view (male) (Dolphin 1987).

❑ Use <u>scissors</u> to lift and cut away one side of the rostrum forward from the anterior cephalothorax Note the **gastric muscles** extending from the rostrum to the stomach and the muscles you cut from the carapace to the stomach. What happens when these muscles contract?

   Mandibular muscles are lateral to the stomach; their contraction causes the mandibles to bite together. Several sets of muscles are responsible for the operation of the gastric mill in the stomach (described below).

❑ Now cut through the exoskeleton covering the abdomen from the first to the last abdominal segments. Carefully remove the exoskeleton to expose the underlying structures. Remove the gills and the membrane separating them from the internal organs. Flood the tray with water so that the organs float.

❑ <u>Digestive system</u>: Trace the digestive system from the **mouth** through the **esophagus** to the **cardiac** and **pyloric stomachs** and into the **intestine.** Find the **anus** at the base of the telson. Locate the large **hepatopancreas** (yellowish mass of tissue) that secretes digestive enzymes into the stomach and stores glycogen. To what vertebrate organs would it be comparable?

❑ The cardiac and pyloric stomachs are thin-walled white structures near the dorsal midline of the cephalothorax. The former is the more anterior; by lifting up its anterior end, the esophagus is easily seen. Cut the esophagus and the intestine to remove both stomachs. Cut open the cardiac stomach and observe and probe the **gastric mill**. The mill consists of chitinous teeth, plates, hairs, and ossicles. It is controlled by some 13 different muscles (several were mentioned above). Carefully wash out the gastric mill with tap water to study its structural organization.

   Only after food has been thoroughly ground in the gastric mill can it pass on to the pyloric stomach. Enzymes from the hepatopancreas are added to the food in the cardiac stomach. When the crayfish molts its exoskeleton, the first three sections of the digestive tract are also shed.

❑ <u>Excretory system</u>: Remove the organs from the cephalothorax to find a pair of large, <u>grayish</u> **green glands** (Figure 7.17 b), which exit through the **renal pore** (Fig. 7.15 b) at the base of the long antennae. These glands function as osmoregulatory organs. Excess water, absorbed through the gill membranes, and nitrogenous waste, mainly in the form of ammonia, are removed from the hemolymph bathing these glands within the body cavity.

❑ <u>Nervous system</u>: Carefully remove any remaining carapace, including the projection between the eyes; avoid injury to underlying tissues. Ventral to the cardiac stomach (which has been removed) locate a pair of large ganglia between the eyestalks. These ganglia constitute the **brain**. Look on the floor of the body cavity to find the **ventral nerve cord**. To see the cord, remove the calcified plates of the ventral cephalothoracic exoskeleton. These plates cover the nerve cord in the thorax. Collections of neurons (**segmental ganglia**) function as integration centers. Lateral nerves leave the ganglia and innervate the appendages.

Are you a lover of lobsters? Again, notice what you are eating the next time you have lobster and enjoy!

Subphylum Hexapoda – the insects

When J. B. S. Haldane was asked by a clergyman what he had learned about the creator based on all his years of study of the products of his creation, Haldane replied that the creator must have an inordinate fondness for beetles. Insects represent about 70% of all described animal species and they are also the most numerous organisms on earth. The Order Coleoptera, which includes the beetles, is the largest group of insects.

The insect body is typically divided into three sections; the **head** (several fused segments), the **thorax** (three segments), and the **abdomen** (12 or fewer segments). The head has various sensory receptors, usually including **compound eyes** and one pair of **antennae**, and a bilobed flap called the **labrum** which covers three pairs of mouthparts (a pair of **mandibles**, **maxillae**, and a **labium,** or lower lip, which is formed from the fused second maxillae). Three pairs of **walking legs** and, usually, two pairs of **wings** are found on the thorax. Appendages on the abdomen are limited to jointed **cerci** (thin sensory projections) and appendages related to mating and egg laying.

The respiratory system of most insects is composed of **tracheal tubes**, highly branched tubes that ramify throughout the body, carrying oxygen directly to the tissues without the use of the circulatory system. The circulatory system is open; arteries discharge hemolymph into sinuses where it bathes various tissues and then recollects in the large pericardial sinus surrounding the heart. Hemolymph enters the heart through one-way valves called **ostia** and is then pumped through arteries back to the tissues. The excretory system of insects usually consists of **Malpighian tubules**. These extensions of the gut project into the fluid-filled body cavity and absorb fluids, concentrate nitrogenous wastes such as uric acid, and conduct urine into the hind gut.

❑	Observe the demonstration dissection of a cockroach (*Blaberus* sp.) to see the tracheal system in a freshly killed insect.

❑	Examine the display of tropical insects in the atrium of Comstock Hall as you leave the laboratory today.

?	Compare and contrast the main mechanisms of locomotion in nematodes, annelids, and arthropods.

Nematodes
Annelids
Arthropods

CONCLUDING ACTIVITIES AND DISCUSSION

❑ Before you dispose of your earthworm and crayfish, your lab instructor will expect you to demonstrate that you have correctly identified all important structures by providing him/her with a "tour" of your specimens.

❑ You should now finish filling in Table 7.1 on the following page to help you organize the material that you have learned about the invertebrates.

❑ Review your understanding of the functions of the various systems for Cnidaria, Platyhelminthes, Nematoda, Rotifer, Mollusca, Annelida, and Arthropoda by reviewing Table 7.1.

POST-LAB WEB ACTIVITIES

To review the dissections you have done and test your understanding of this laboratory, study the associated web materials located in the lab section of the course web site.

URL = http://biog-101-104.bio.cornell.edu/BioG101_104/tutorials/animals.html

You will encounter questions similar to these on major quizzes and the practical examination.

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Table 7.1. The development of invertebrates structural complexity.

FEATURES: (List organisms studied in lab)	BODY SYMMETRY	TISSUE LAYERS PRESENT	CONDITION OF COELOM	DIGESTION & EXCRETION	CIRCULATION	RESPIRATION	NERVOUS & SENSORY	SKELETON & LOCOMOTION
CNIDARIA:								
PLATYHELMINTHES:								
NEMATODA:								
ROTIFERA:								
MOLLUSCA:								
ANNELIDA:								
ARTHROPODA:								

NOTES

CHAPTER 8 – ANGIOSPERM GROWTH AND DEVELOPMENT

LABORATORY SYNOPSIS

In the first week of this two week laboratory sequence you will begin two studies.

1. You will make initial measurements and treatments on dwarf pea plants in a bioassay for gibberellins extracted from the growth medium of a fungus.
2. You will make initial observations on germinating corn and bean seeds and then plant them for studies in the second week.

In the second week you will finish and discuss the gibberellin bioassay. Then you will study the morphology and physiology of corn and bean seeds beginning the germination process, and observe seedling structures which originated in and developed from the components of the plant embryos. You also will observe and discuss a demonstration showing the effect of light on plant development.

Please bring a calculator to both laboratory periods, and know how to calculate standard deviation with your calculator.

Below is a schedule for the two laboratory periods:

Week 1: (Monday, July 26, 2010)
1. Start the gibberellin bioassay.
2. Plant germinating seeds.

Week 2: (Monday, August 2, 2010)
1. Complete the gibberellin bioassay.
2. Complete study on seed and seedling morphology.
3. Observe seedlings showing the effect of light and darkness on their development and morphology.

LABORATORY OBJECTIVES

Conceptual Objectives

1. Know the origin of the embryo, endosperm and seed coat of a seed.

2. Know the major differences between monocot and eudicot angiosperms with respect to the morphology of their embryos.

3. Know the main parts of a corn and bean seed, the major morphological differences between these seeds, and the roles of the seed structures in the development of a plant embryo into a "plant."

4. Know the major parts of a corn and bean seedling and the origin of these parts in the embryo.

5. Understand the concept of a "meristem" and how meristems are involved in the primary growth of a plant.

6. Know the difference between epigeous and hypogeous seed germination.

7. Know the major differences in morphological development between dark-grown and light-grown seedlings.

8.   Know the origin of a branch in the shoot system of an angiosperm and concept of apical dominance in angiosperms

9.   Know what a bioassay is, understand the methods for making and using a dose-response curve and understand the meaning of a "threshold dose" and a "saturation dose."

10.   Understand how the class-conducted bioassay used a paired design to reduce variation.

Procedural Objectives

1.   Know how to use the appropriate data to construct a dose-response curve and how to use the curve in a bioassay.

2.   Know how to calculate the mean and standard deviation of a data set.

READING ASSIGNMENTS (should be done <u>before</u> lab period)

For Week 1:

> In this chapter
>> Introduction: Week 1   p. 166-170
>> Gibberellin Enhanced Growth of Dwarf Pea Plants
>> A Bioassay for Gibberellin Using Primary Growth of Dwarf Peas
>>> (Introduction: Background Information)
>> Plant seeds for growth study   p. 175

For Week 2:

> In this chapter:
>> Introduction: Week 2   p. 170-182

Descriptive statistics from the Statistical appendix in the BioG 1107 lab manual.

In Campbell and Reece, *Biology*. 8th ed. (2008):

Chapter 38:   Concept 38.2   p. 812-815

Chapter 39:   Concept 39.1- 39.4   p. 821-845

                                                                              Paul R. Ecklund

                                                                              Revised June 2010
                                                                              Mark A. Sarvary
                                                                              Scott Meissner

QUESTIONS TO PREPARE YOU FOR THIS LABORATORY

For Week 1:

1.   A.   What is the <u>genetic</u> difference between the tall and dwarf pea plants we will use in this laboratory?

      B.   How does the genotype of dwarf pea plants cause them to be shorter than "tall" pea plants?

2.   What is a bioassay?

3.   What kind of organism produced the gibberellin you will use in your bioassay?

4.   You will be using two variables in your bioassay.

      A.   What is the independent variable?

      B.   What is the dependent variable?

For Week 2:

1.   Can you calculate a <u>mean</u> and a <u>standard deviation</u> for a set of data with your calculator? Please learn how to do this before lab. For additional help, please see Appendix 4, Statistical Reference, in the 1107 Manual.

2.   A.   What is the origin of endosperm in a seed?

      B.   What is the function of endosperm in a seed?

3.   A.   A seed develops from what floral structure?

      B.   A seed coat develops from what part of that floral structure?

      NOTE: You should know the answers to questions 2 and 3 from last week's laboratory.

WRITING ASSIGNMENT

At the end of this chapter is a question regarding the growth and development of a plant embryo into a seedling. You are required to write <u>individually</u> a complete and concise answer, which should summarize your understanding of several concepts treated in this laboratory.

This assignment is due in your laboratory period on Wednesday, August 4, 2010.

Please type your answer using 12 point font and limit your answer to one page, double-spaced.

INTRODUCTION: WEEK 1

The integration and coordination of processes involved in the growth and development of a whole plant are regulated by a variety of substances. Six major kinds of plant "hormones" are generally recognized: auxins, gibberellins, cytokinins, ethylene, brassinosteroids and growth inhibitors (abscisic acid and perhaps others). Hormones are organic molecules made in one location, then moved through the organism and affect certain cells elsewhere. The term "auxin," "gibberellin," or "cytokinin" is a generic name referring to a group of substances that cause the same physiological responses and are similar in chemical structure. For example, more than 120 different substances are known as gibberellins, based on similarities in their chemical structures. Many of these gibberellins are biologically active in that they elicit a physiological response in a plant system; however, some gibberellins appear to have no biological activity.

This laboratory sequence focuses mainly on the gibberellins, which seem to be involved in all stages of plant growth and development from seed germination to flowering and fruit development. Special attention will be given to the role of gibberellins in the primary growth (elongation) of shoots. The involvement of this group of growth regulators in seed germination also will be briefly considered in the second week.

Many herbacious angiosperms produce a shoot consisting of a single stem and leaves with few or no branches. Nevertheless, at the base of each leaf, where it attaches to the stem, is a bud which is capable of growing and developing into a branch. **Apical dominance** is a phenomenon in which the tip of the shoot is able to suppress the growth of these buds and maintain a single stem with little or no branching. An auxin produced by the apical bud moves down the stem and inhibits the growth of the lateral buds. You will study this role of auxin by excising the shoot tips from several pea plants and then applying a lanolin paste with or without an auxin to determine the effect of these treatments on the development of lateral buds into branches.

GIBBERELLIN ENHANCED PRIMARY GROWTH OF DWARF PEA PLANTS

Dwarfism is occasionally encountered in plant species. If it is due to a genetic mutation, it can be perpetuated in a "strain" or "variety" of a species by selective breeding. The dwarf variety of pea (*Pisum sativum*) that you will use in this study was obtained in this way. Several different genes (loci) are known to determine stem length in peas. The homozygous recessive condition for any one of those genes results in a dwarf plant (recall Mendel's studies with tall and dwarf peas). Plant dwarfism usually is the result of reduced elongation of internodes (sections of stem between leaves; see Figure 8.2.A). In many dwarfed plant varieties the application of a gibberellin causes the plants to develop as "normal" tall plants, suggesting that the dwarfism is due to a block in gibberellin production in the plants. The stem length gene that Gregor Mendel studied is designated the *Le* gene in peas. Plants with the genotypes *Le/Le* or *Le/le* are tall (normal), while those with the *le/le* genotype are dwarfed.

Presently more than 120 different gibberellins have been found in plants and fungi. Each is numbered and given the abbreviation GA (for example, GA_5). Both tall and dwarf varieties of pea plants produce approximately a dozen different GAs; however, recent research has established that dwarf peas with the genotype *le/le* are deficient in GA_1, which is found in greater amounts in the tall varieties. In peas the immediate precursor of GA_1 is GA_{20}, which is hydroxylated by an enzyme, GA 3b-hydroxylase, to make GA_1. Dwarf *le/le* pea plants have very low GA 3b-hydroxylase activity compared to that in tall plants. The application of GA_1 to *le/le* dwarf peas enables them to grow like normal plants.

In 1996-97 a group of researchers (Lester and others 1997) used molecular biological technology to isolate and clone the *Le* and *le* alleles of the *Le* gene of peas and analyze the GA 3b-hydroxylase coded by each allele. cDNAs of both alleles were prepared from mRNA and were ligated into plasmids. *Escherichia coli* was transformed with either the plasmids containing the *Le* cDNA or plasmids with the *le* cDNA. Transformed *E. coli* cells were grown in culture to increase the number of cells and the amount of GA 3b-hydroxylase produced by the cells, then they were lysed and the lysate (cell contents including the GA 3b-hydroxylase) was assayed for its ability to convert GA_{20} to GA_1. Lysate from *E. coli* carrying the *Le* cDNA had 20-fold greater GA 3b-hydrolyase activity than that in the lysate from *E. coli* with the *le* cDNA. The nucleotide sequences of the *Le* and *le* alleles were determined. According to the sequence data, the only difference between the predicted proteins coded by the two alleles is that the *le*-coded protein has threonine in a position where the *Le*-coded protein has alanine. This amino acid substitution occurs at a position assumed to be near the enzyme's active site and has a profound effect on the enzyme's ability to convert GA_{20} to GA_1. The fact that *le/le* dwarf peas grow by stem elongation indicates that some GA_1 is present in them, but apparently their GA 3b-hydroxylase does not produce sufficient GA_1 for a "normal" growth rate.

The dwarf variety of *P. sativum* you will use is homozygous for the *le* allele. No other gibberellin produced by peas can substitute for GA_1 and enhance shoot growth in dwarf peas. But, some gibberellins made by other organisms can substitute for GA_1. For example, GA_3 is similar enough in chemical structure to GA_1 that it produces the same physiological effects. The two compounds differ only by the presence of a C-C double bond in one ring of GA_3 (see Figure 8.1).
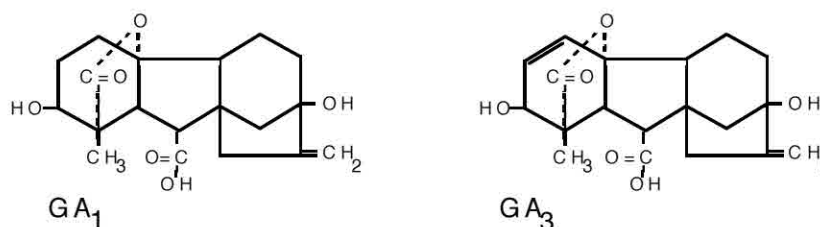
Figure 8.1. Chemical structures of GA_1 and GA_3. Each angle in a ring represents a carbon atom with the appropriate number of bonded hydrogen atoms. The broken lines represent bonds in a ring whose plane is perpendicular to the plane of the page.

Although GA_3 is produced by some plants, it is not produced by peas. The main commercial source of GA_3 is a fungus, *Gibberella fujikuroi*, which synthesizes and secretes the substance in large amounts. You will measure the primary growth response of dwarf pea plants to different concentrations of GA_3.

A BIOASSAY FOR GIBBERELLIN USING PRIMARY GROWTH OF DWARF PEAS

Introduction: Background Information

A bioassay is a procedure that enables one to determine the presence of a substance by the response of a biological system to the substance. The biological systems used range in complexity from individual cells to complete multicellular organisms. Bioassays can be used to detect and estimate concentrations of such substances as hormones, pheromones, vitamins, plant growth regulators, and even pollutants.

A qualitative bioassay is used merely to determine whether or not a certain type of substance is present in a test solution, homogenate, or gas. A quantitative bioassay is used to estimate the amount of a substance present in the medium tested, and requires the response of the biological system to be related to the amount of the substance causing the response. Routinely in a quantitative bioassay, samples of the biological system are exposed to a range of known amounts of the substance being assayed to obtain a **dose-response curve**. Additional samples of the biological system are treated with the medium in which the amount of the assayed substance is unknown, and the resultant responses are compared to responses on the dose-response curve.

In addition to the seven known concentrations of GA_3 you will use to obtain a dose-response curve, you will use three different concentrations of an extract from a fungus culture medium in your bioassay. Each solution will be applied to a group of plants (a different group for each solution) and the growth response of plants in each group will be determined. The average growth response of groups treated with different concentrations of the extract will be "fitted" to the dose-response curve to obtain an estimate of the gibberellin concentration in the extract.

Morphology of the Pea Plant

An understanding of the morphology of the pea plant is essential for conducting this study. Tall and dwarf pea plants have the same morphology; tall plants have longer sections of stem between leaves.

❑   Select a pot of plants, carefully examine a plant, and identify the structures and regions shown in Figure 8.2

At the base of the stem, on some plants just below the potting medium, is a spherical structure (it may be split into two hemispheres). These two hemispheres are the **cotyledons**, or embryonic leaves, of the seed. The stem is divided into **nodes** (sites of leaf attachment) and **internodes** (sections between nodes). The **cotyledonary node** is a reference point for numbering all subsequent nodes and for measuring stem height. Usually the first two leaves above the cotyledons are quite small; each consists of only a single blade. The third and subsequent nodes above the cotyledons have complete leaves, each consisting of two **stipules** (ear-shaped structures at the base of the petiole), a **petiole with two leaflets**, and a **tendril** (see Figure 8.2.D). In Figure 8.2.A, leaflets and stipules are numbered according to their node numbers above the cotyledons. The **highest visible node** is the one with immature stipules appressed to each other and leaflets which are not fully opened (node 5 in Figure 8.2.A).   The appearance of the leaf at the highest visible node varies among plants. On some plants the leaflets may be quite small and folded between the closed stipules (Figure 8.2.B); on others the leaflets may be nearly mature and partially opened (Figure 8.2.C). If you carefully separate the closed stipules you can see a smaller immature leaf–the next to appear as the plant grows. The site where the midveins of the closed stipules attach to the stem is the location of the **highest visible node**, an important reference point for measuring stem height. Two-week-old pea plants generally have four or five visible nodes above the cotyledonary node.

❑   Compare the morphology of tall and dwarf pea plants of the same age. Please do not handle the tall plants; they are more delicate than dwarf plants and must be used in subsequent laboratories.

Bioassay Procedure

Ten containers with 8-10 16-day-old dwarf peas are available.

❑   Work in pairs; each pair of students does the following:
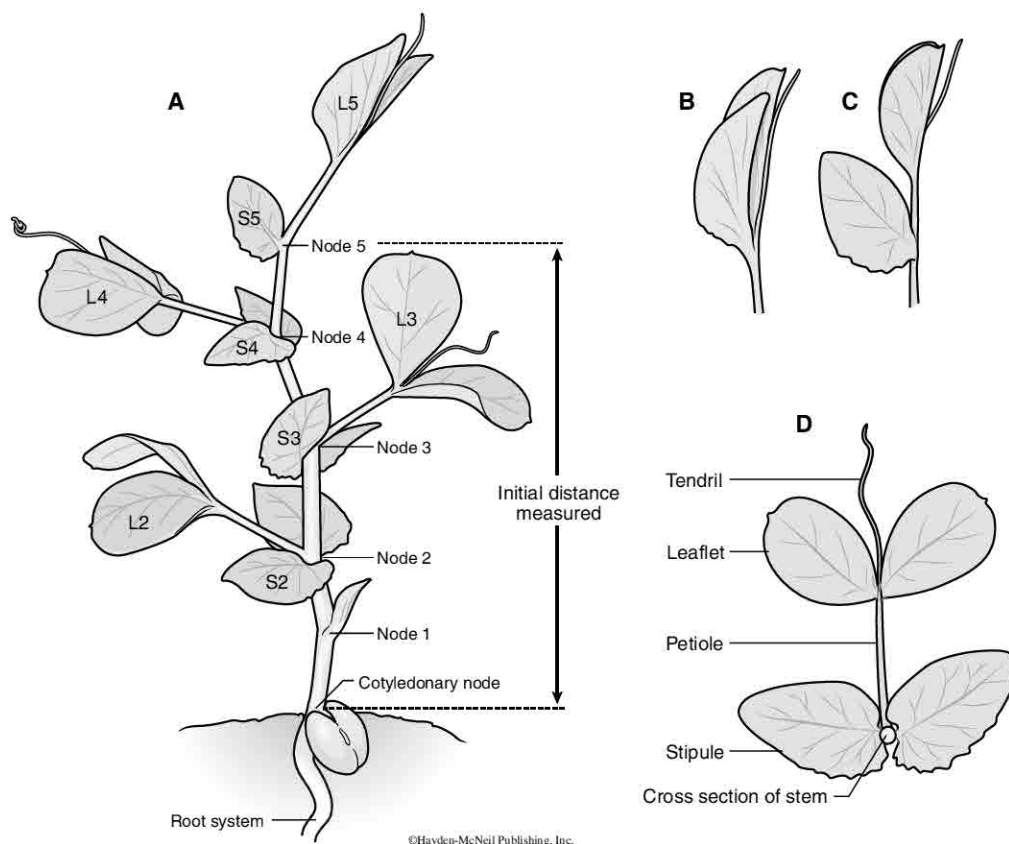
Figure 8.2. A: Diagram of a two-week-old dwarf pea plant, x1. One leaflet (L) and one stipule (S) of each pair on a leaf are labeled for each node. B and C: Diagrams showing variation in appearance of the immature leaf at the highest visible node, x1. D: Diagram of a complete pea leaf showing its components, x1.

Week 1 Procedure

1. Select a container of plants and one of the treatment solutions -- six known concentrations of GA_3, a control (no GA_3), and three concentrations of the extract.

2. With tape and a pencil (ink may wash off), label the plant container with the following information:

   Your names
   Lab day and time
   Lab room number
   Treatment (written on treatment solution's container)

3. Prepare a small flag of masking tape on a toothpick for each plant in your tub. Number the flags consecutively with a pencil (ink may wash off). Label each plant by pushing a flag's toothpick deeply into the growth medium beside each plant. Only the tape of each flag should be above the surface of the growth medium.

4. Refer to Figure 8.2. Be sure you can identify on your plants the parts labeled in the figure. Your plants should have four or five visible nodes above the cotyledonary node. Note that the third node above the cotyledons is the lowest node having a compound leaf with leaflets; knowing this will facilitate collecting data.

Note: Determining the position of the **highest visible node** may be difficult, because the youngest visible leaf is oriented vertically as if it were part of the stem. Be sure you measure stem length to the base of the youngest leaf and not to some position on the leaf. If you have difficulty locating the highest visible node, ask your instructor for assistance.

5. Leaving the plants intact in their growth medium, and handling them carefully to avoid damaging them:

    a.   Measure on each plant and record in one copy of Table 8.1 the length of the stem in millimeters from the **cotyledonary node** to the **highest visible node** (see Figure 8.2.A).

    b.   Count on each plant and record in Table 8.1 the number of visible nodes above the cotyledonary node.

    Note: If a plant's stem is broken, consult your lab instructor about discarding the plant. Be sure to omit data obtained from a discarded plant.

6. Partially separate (carefully) the appressed stipules at the highest visible node of each plant and apply one drop of the treatment solution between the stipules with a dropper. The closed stipules should hold the drop of liquid in place; however, if the drop falls off the plant, apply another one. Be sure every plant in the group is treated.

    You may be asked to measure and treat a second group of plants. Record the data for the second group in the second copy of Table 8.1.

    Note: Both members of a pair should record the data for each treatment they use. If one member is absent from lab the following week, the other member will have the data to complete the study.

Week 2 Procedure

7. Repeat step 5 of the previous week's procedure. To facilitate measuring you may remove the shoots from the pot by cutting each plant below its cotyledons.

8. Complete Table 8.1, including the calculation of the mean and standard deviation for % change in stem length and number of nodes added. Record your data on the chalkboard and complete Table 8.2.

9. Plot the appropriate data from Table 8.2 in Figure 8.3.

10. Estimate the amount of GA_3-like substances present in one drop of each of the extract solutions by putting a point which corresponds to the mean response obtained from each extract solution on the dose-response curve, and then determining the dose of GA_3 with which each point corresponds.

Discussion and Interpretation of Results from the Studies on Plant Growth Responses to Gibberellins and the Bioassay

Notice that the abscissa of Figure 8.3 is broken in the increment between zero and 0.001 μg GA_3. This is done routinely to indicate that the difference between these two doses is infinite compared to the finite and uniform difference between any other two consecutive doses. The dose-response curve also should be broken in the zero to 0.001 increment of the graph.

Table 8.1. Data from a single treatment group in the gibberellin-induced growth study.

Treatment: _____ μg GA$_3$/drop

Replicate number	Initial stem length (mm)	Final stem length (mm)	% Change in stem length*	Initial number visible nodes	Final number visible nodes	Number of nodes added
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
		Mean			Mean	
		Standard deviation			Standard deviation	

Treatment: _____ μg GA$_3$/drop

Replicate number	Initial stem length (mm)	Final stem length (mm)	% Change in stem length*	Initial number visible nodes	Final number visible nodes	Number of nodes added
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
		Mean			Mean	
		Standard deviation			Standard deviation	

* % Change in stem length = $\dfrac{\text{Final stem length - Initial stem length}}{\text{Initial stem length}}$ x 100

Table 8.2. Growth response data and related data from different treatment groups of dwarf *Pisum sativum*.

| Parameter | μg GA$_3$/plant | | | | | | | | | |
	0	0.001	0.01	0.1	1.0	10	100	Ext.	Ext. 1/10	Ext. 1/100
Mean % change in stem length										
Std. Dev. of % change in stem length										
Mean number of nodes added										
Std. Dev. of number of nodes added										
Initial number of plants										
Final number of plants										

?   The plants treated with 0 μg GA$_3$ were the control group for the experiment. Were there any doses of GA$_3$ which produced a mean growth response <u>no greater</u> than that of the control group?

If so, which?_____ How would you verify your answer?

In physiology the term **"threshold"** is used to describe a point or value below which no effect is produced and above which an effect is produced.

?   What is the best estimation of a threshold GA$_3$ dose for growth enhancement in dwarf peas in your experiment? _____

Recall from your study of enzyme action that when an enzyme-catalyzed reaction is "saturated" with substrate, no increase in reaction velocity occurs with the addition of more substrate. The principle of "saturation" may also be applied to hormone-promoted responses. The response system is saturated with the hormone when the response does not increase with the application of greater amounts of the hormone.

?   What is the lowest dose of GA$_3$ that "saturates" the system(s) involved in the plants' growth response? _____

In many hormone-induced responses, after the hormone threshold is exceeded the response is linearly related to the logarithm of the hormone concentration, which means that lower doses of a hormone are relatively much more effective than higher ones. This may be explained in the following way. Many hormones are known to attach to specific receptor sites on or in certain "target cells." The binding of hormone molecules with receptor molecules initiates a series of reactions which ultimately result in the response(s) of the biological system to the hormone. It is generally assumed that the molecules of any substance with hormone-like activity start their effect by attaching to specific receptor molecules. The

probability of a hormone-receptor interaction occurring depends on the concentrations of both hormone and receptor molecules. The portion of the biological system that is "receptive" to the hormones has a finite number of receptors. As more and more receptors bind hormone molecules, the probability of encountering an available receptor decreases; consequently the hormone concentration must be greatly increased to increase the chances of hormone-receptor encounters. A relatively very high concentration of hormone molecules is required to saturate the receptors just as a very high concentration of substrate molecules is needed to saturate a given number of enzyme molecules. However, it is important to remember that several biochemical and physiological processes may be involved in producing the response that was initiated by hormone-receptor binding. Factors affecting any of these intermediate processes would affect the apparent relationship between hormone dose and response.

The "mechanism of action" of a hormone is the event involving the hormone and its receptor that initiates the response produced by the hormone. Studies on gibberellin's mechanism of action in grain seed germination have shown that gibberellin binds to a plasma membrane receptor with a G protein transducer. The activated G protein causes the formation of the second messenger cyclic GMP which indirectly promotes the transcription of a gene coding for the starch digesting enzyme, alpha amylase. Presumably gibberellin's mechanism of action in promoting stem growth also is its attachment to a receptor with a G protein transducer, but the subsequent reactions initiated by the GA-receptor complex are not understood.
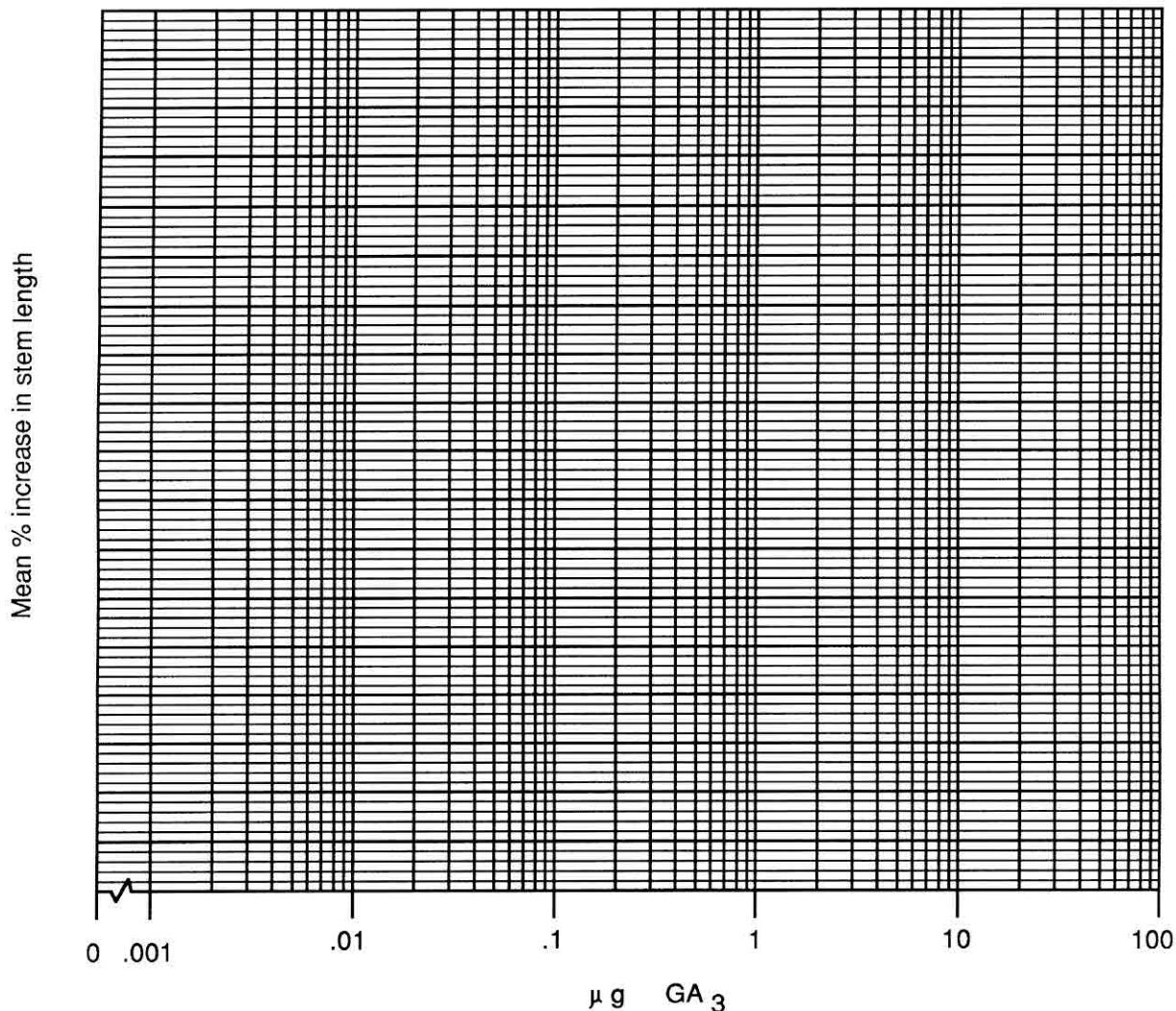
Figure 8.3. The growth response (mean ± SD) of dwarf *Pisum sativum* to different doses of GA_3.

The concentration of response-producing substances in the assayed ("unknown") medium determined by a bioassay is generally expressed in terms of the substance used to obtain the dose-response curve. For example, the concentration of growth-promoting substances in the extract used in your bioassay should be expressed as μg **GA$_3$-like substances**/drop or μg **GA$_3$ equivalents**/drop. The extract may contain several growth-promoting substances, including GA$_3$. By expressing the amount of growth-promoting substances in terms of GA$_3$ equivalents, one indicates that all the growth-promoting substances, collectively, in a given volume of solution are equivalent, in the response they elicit, to a certain amount of GA$_3$ in the same volume of solution.

?   What was the estimated amount of GA$_3$-like substances present in one drop of each of the extract concentrations you bioassayed?

Undiluted extract: _____; 1/10 dilution: _____; 1/100 dilution: _____

?   Why were three concentrations of the extract used in the bioassay?

?   Was the gibberellin-induced growth of dwarf pea plants mainly the result of the production of more nodes, the greater elongation of internodes, or both? (Provide evidence for your answer.)

?   Bioassay Problem:

One megagram (10^6 grams) of fresh hydrated pea seeds was homogenized and extracted with an organic solvent to remove the gibberellins. The organic solvent with dissolved gibberellins was filtered to remove cellular debris. By a vacuum evaporation process the organic solvent was evaporated away, and the remaining residue, containing the gibberellins, was dissolved in a sufficient volume of an ethanolic solution to make a final volume of 10 ml. This ethanolic solution of gibberellins was bioassayed by the same procedure you used. The estimated concentration of GA$_3$-like substances in the solution was 0.3 μg/drop.

Assuming that 1 drop = 0.05 ml, and that no GA$_3$-like substances were lost in the extraction and concentration procedures:

1.   What was the total amount of extractable GA$_3$-like substances present in the megagram of seeds?

2.   The average weight of a fresh pea seed is 0.5 g. What is the amount of extractable GA$_3$-like substances present in a single seed?

SEED MORPHOLOGY AND EMBRYO DEVELOPMENT

Planting Seeds to Make Seedlings for Next Week

Next week you will study the morphology of bean and corn seeds that are just beginning **germination**, the resumption of growth by the formerly dormant embryo. So that you may see the extent of growth and development that occurs during one week as an embryo develops into a seedling, you need to plant germinating seeds this week so you will have seedlings to observe next week.

Dry corn and bean seeds were placed on water-soaked paper towels two or three days ago. The seeds imbibed water and their embryos have begun growing. The first structure to emerge from the seed is the embryonic root, or **radicle**.

❑ Work in pairs.

❑ Each pair obtains one tub of water saturated vermiculite. Keep the tub on a plastic plate.

❑ Label the tub with a masking tape label having group members' names (or initials), lab day and time on it.

❑ Thoroughly moisten a paper towel with water, then obtain 3 corn seeds and 3 bean seeds from the germination trays and place them on the wet towel. Observe them and note a radicle (small, white, tapered structure) emerging from the middle of each bean seed or from the narrower end of each corn seed. A coleoptile also may be emerging from the corn seed (see Figure 8.7).

❑ In the space below make a sketch of a germinating bean and corn seed showing the emerging structures.

❑ Evenly distribute the six seeds on the surface of the vermiculite in the tub. Take care to not break the delicate radicle.

❑ Cover the seeds with a layer of dry vermiculite level with the top of the tub, and return the tubs (without the plates) to the plastic tray.

The tubs will remain in your lab room and be irrigated as needed until next week, when you will remove the seedlings from the vermiculite and study their morphology.

INTRODUCTION: WEEK 2

A preceding laboratory and its associated lectures addressed the morphology and function of flowers, various methods of accomplishing pollination in the flowers and the unique double-fertilization process which follows pollination. This laboratory addresses the product of pollination and fertilization—the embryo. You will study plant embryos in seeds, and follow the development of embryos into young plants as they build roots and shoots by primary growth. You also will observe the effects of the presence and absence of light on the growth of young plants.

Only two groups (clades) of Anthophyta will be considered: eudicots and monocots. A eudicot has two cotyledons, or embryonic leaves in its embryo; a monocot embryo has one cotyledon. Monocots and eudicots also differ in flower and leaf morphology; you observed this in a preceding laboratory.

The embryo in a mature angiosperm seed is a relatively simple structure. In many species it consists of no more than one or two colyledons attached to an elongate structure that gives rise to the root and shoot. At each end of the root-shoot structure is an **apical meristem**. **Meristems** are regions of continual cell division. Linear growth of the embryo into a plant and continual linear growth of that plant is accomplished by the activities of apical meristems—one in each shoot tip and one in each root tip (see Figures 8.4 and 8.5). By definition, **primary tissues** are those that are derived directly from apical meristems. Thus, linear growth is **primary growth** and involves elongation of the root and its branches, elongation of the stem and its branches, and the formation and enlargement of leaves and buds.

Many herbaceous plants and all woody perennial plants have meristems that enable their roots and stems to grow laterally (increase in width). Lateral growth resulting from meristematic activity is called **secondary growth**. In all species exhibiting secondary growth an increase in width results mainly from the production of more vascular tissue. The stems and roots of woody perennial plants live for many years and their circumferences increase each year. This necessitates the formation of additional surface tissue to cover the increasing outer surface. The formation of periderm or "outer bark" on woody perennials also is secondary growth which results from the activity of a meristem near the surface of the stem or root.

Secondary growth in plants will not be considered in this laboratory.

SEED MORPHOLOGY AND EMBRYO DEVELOPMENT

Structure of the Seed

The seed is a structure produced only by angiosperms (flowering plants) and gymnosperms (naked-seeded plants such as conifers). A seed consists of a **plant embryo**, **stored nutrients** for the development of the embryo, and a protective covering called the **seed coat**. In a dried, viable seed, growth of the embryo is suspended, and the seed is considered to be <u>dormant</u>. The seeds of many species require merely the absorption of water to break dormancy and initiate <u>germination</u>, the resumption of growth by the embryo.

❑   Work in pairs on this exercise. Each person needs a dissection microscope; each pair needs beaker containing a small volume of water.

<u>Bean</u> <u>(Eudicot)</u> <u>Seed.</u>

❑   Each person obtain and examine a seed of bean (*Phaseolus vulgaris*) which has been in water for several hours. Carefully remove the seed coat, or testa, the thin outermost covering. Note that with the testa removed the seed appears to consist of two halves that are joined at one point. Carefully separate the two halves and observe the seed, with the insides of the halves exposed, under the stereoscopic microscope.

leaf
primordia

lateral bud
primordia

developing
vascular tissue
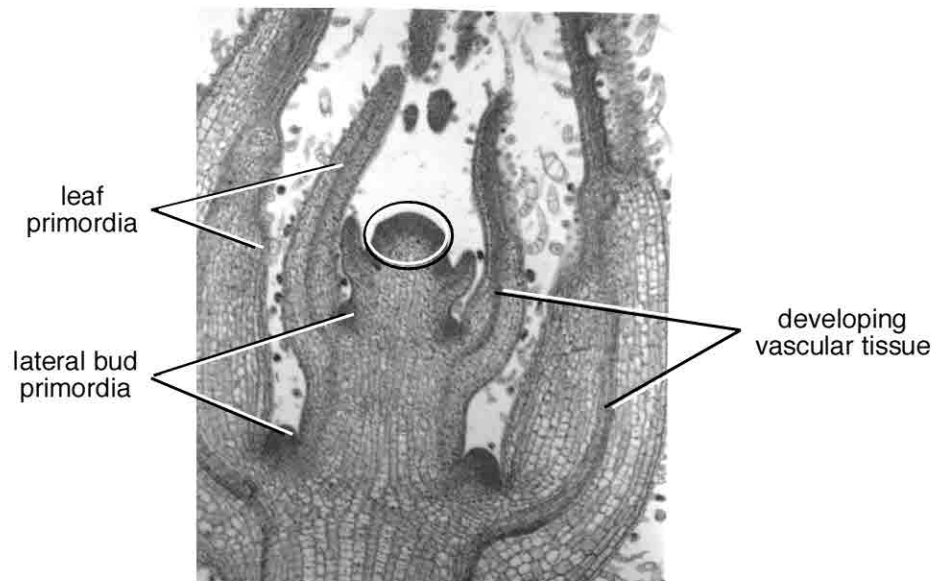
Figure 8.4. Longitudinal section of a Coleus shoot tip. The ring indicates the apical meristem. All the cells of the primary stem, the leaves, and the lateral buds are derived from the apical meristem. Primordia are immature structures. Each lateral bud, when mature, will possess an apical meristem, and under appropriate conditions will develop into a shoot system like this as a branch from the main stem.

root cap
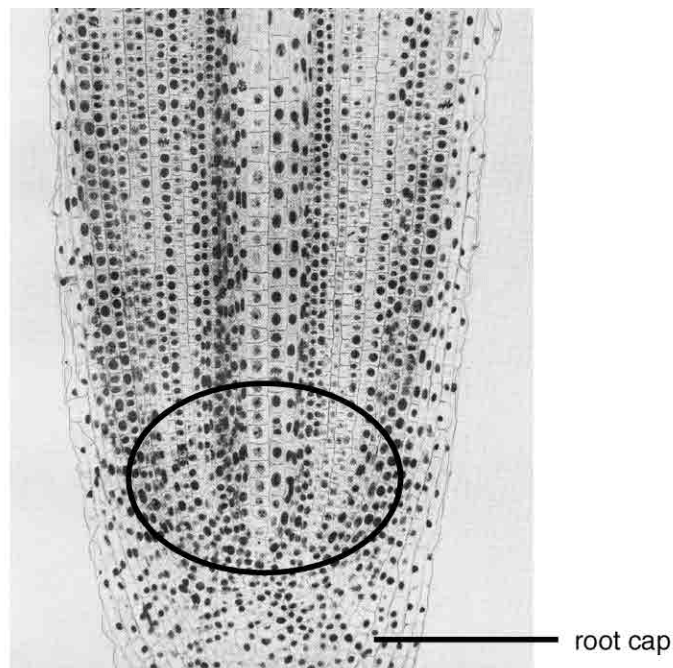
Figure 8.5. Longitudinal section of an onion (*Allium cepa*) root tip. The ring indicates the apical meristem, a region of continual cell division. All the cells seen here and those further up the root are derived from the apical meristem. This photomicrograph shows that portion of the root in which most of the cell divisions are occurring. Immediately above this portion is a region of cell elongation, and above that is a region of cell differentiation where root hairs are formed. The root cap is the dome-shaped structure composed of large cells below and beside the apical meristem.

The entire structure you are observing is a plant embryo. The two "seed halves" are **cotyledons**, or embryonic leaves containing stored nutrients. The "hinge" between the cotyledons is the **root-shoot structure**, with an apical meristem at each end. See Figure 8.6. Cell divisions in these meristems and subsequent enlargement and differentiation of cells derived from these meristems result in the development of the root-shoot system of the plant (see Figures 8.4 and 8.5). Notice that in the mature bean seed the two primary leaves of the plant shoot were formed before the seed became dormant. This degree of shoot development in the seed is not typical of seeds of all species. The function of the cotyledons in a mature bean seed is to provide nutrients and growth-regulating substances to the developing root-shoot structure. As the root and shoot grow, the cotyledons diminish in size and eventually die.

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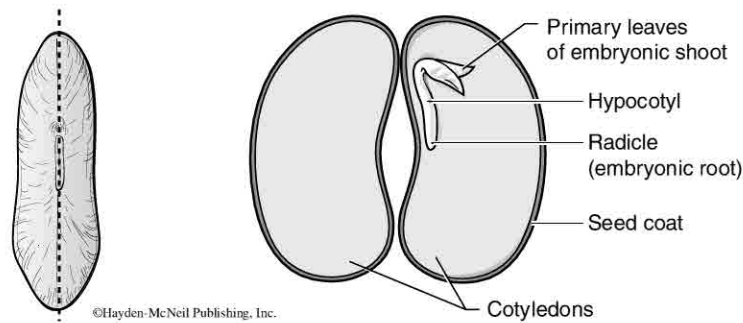
Figure 8.6. Bean seed with cotyledons separated to show the embryonic axis.

❑   Place the seed halves on a wet paper towel, cover them with the towel and keep them for a subsequent procedure.

Corn (Monocot) Seed.

❑   Each person obtain and examine a seed of corn (*Zea mays*) which has been in water for several hours. Notice a white, somewhat oval-shaped region on one of the broad sides.

This white region is one side of the **embryo** (see Figure 8.7.A). For a more "in depth" view of the seed do the following:

❑   Using a sharp razor blade, make a median longitudinal cut through the seed in the plane of bilateral symmetry, as shown by the dashed line in Figure 8.7.B. Examine the seed's cut surface with the stereoscopic microscope and attempt to identify the structures shown in Figure 8.7.C.

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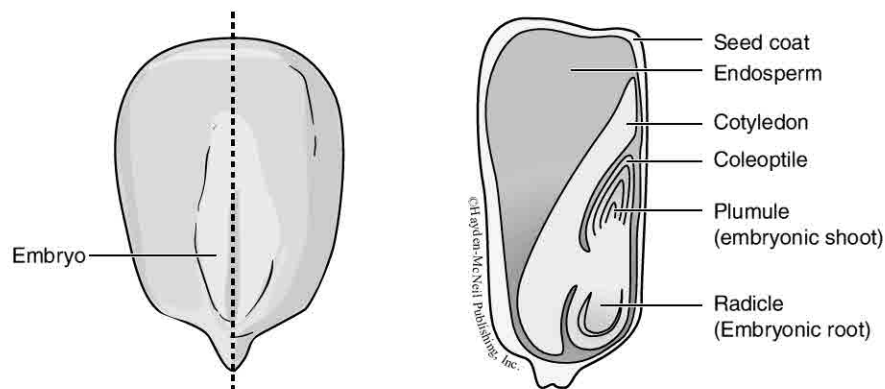
Figure 8.7. Views of corn seed, x4. A. Surface view showing embryo. B. Surface view showing plane of median longitudinal cut (dashed line). C. Median longitudinal view showing components of the seed and embryo.

❑   Both members of a pair do this procedure: Select the better one of your corn seed halves and your bean cotyledon <u>with</u> <u>the</u> <u>attached</u> <u>root-shoot structure</u>.

❑   One pair member do this procedure: Submerge the corn seed half and the bean cotyledon in an iodine-potassium iodide solution for 1-2 minutes (or until a dark blue-purple color develops in some of the tissues). Rinse the seed half and the bean cotyledon with water and prepare them for microscopic observation.

❑   The other pair member do this procedure: Submerge the corn seed half and the bean cotyledon in a triphenyltetrazolium chloride solution for about 15 minutes or longer (until some of the tissues become red). Rinse the seed half and bean cotyledon with water and prepare them for microscopic observation.

❑   Both pair members observe the stained seed halves and record the results of the staining procedures in Table 8.4.

Table 8.4. Components of corn and bean seeds stained with iodine-KI and triphenyltetrazolium chloride (TTC) solutions. Staining is indicated by a +; no staining is indicated by a zero (0).

Seed type	Seed part	Stained in:	
		Iodine-KI	TTC
Corn	cotyledon		
	endosperm		
	embryonic axis		
Bean	cotyledon		
	embryonic axis		

❑   Direct your attention to the stained corn seed halves.

The different structures of the corn seed now should be quite obvious. In the iodine-stained seed half, the **embryo** is stained lighter than the bulk of the seed which is **endosperm**, a nutritive tissue used by the developing embryo. Iodine stains starch blue-purple; the intensity of the staining is proportional to the starch concentration. Obviously, endosperm has a high starch content. The single **cotyledon** (often called a "scutellum") of the embryo is a shield-shaped structure which absorbs nutrients from the endosperm and transfers them to the developing root-shoot structure. Notice that the cotyledon also contains starch.

❑   Use Figure 8.7C to identify the components of the corn seed's root-shoot structure which consists of a **radicle** (embryonic root) with a well defined root cap, and a **plumule** (embryonic shoot) surrounded by a sheath, the **coleoptile**.

?   What is the origin of endosperm?

?   How do the nuclei of endosperm cells differ from the nuclei of embryonic cells?

?   Which part of the corn seed (embryo or endosperm) has the greater intensity of red in the triphenyltetrazolium chloride-treated seed half?

_____

Triphenyltetrazolium chloride, which is soluble and colorless in aqueous solution, can be reduced (by addition of H atoms) to a bright red, insoluble substance, triphenyl formazan. Triphenyltetrazolium chloride can be used to demonstrate the intracellular localization of dehydrogenase enzymes because: 1) it penetrates into cells, 2) it does not inhibit enzyme activity, 3) its reduction product, triphenyl formazan, is bright red and insoluble, so it precipitates at the site of enzyme activity. All dehydrogenases will reduce triphenyltetrazolium chloride *in vivo* by using cellular metabolites as hydrogen sources.

Recall that glycolysis and aerobic respiration involve several oxidation-reduction reactions catalyzed by dehydrogenases. Normally coenzymes such as NAD^+ and FAD receive the hydrogen atoms removed from substrate molecules by the dehydrogenases, but when triphenyltetrazolium chloride is present, it gets the hydrogen atoms and is reduced to the insoluble, red triphenyl formazan. Unlike NADH or $FADH_2$, triphenyl formazan does not transfer its hydrogen atoms to other molecules.

The major constituents of endosperm cells are starch and proteins, macromolecules which are hydrolysed to smaller molecules which, in turn, are absorbed by the embryo for further metabolism.

?   Would you expect dehydrogenase activity in the hydrolysis of starch and protein?

?   Assuming that much of the dehydrogenase activity in the corn embryo is from enzymes involved in glycolysis and respiration, why are these enzymes so active in the growing tissue of the embryo?

The role of gibberellin in corn seed germination

In grains, such as corn, wheat, oat, barley, etc., the endosperm of the seed is surrounded by a thin tissue called the aleurone layer. When the seed becomes hydrated, gibberellin is produced by the embryo and moves into the aleurone layer. In the aleurone tissue, gibberellin induces the synthesis of enzymes including amylases and proteinases which are secreted into the endosperm. These enzymes catalyze the hydrolysis of starch and proteins to the smaller molecules of maltose and amino acids, respectively, which can be absorbed by the cotyledon of the embryo. See Figure 39.11 on page 831 of Campbell and Reece (2008).

❑   Direct your attention to the stained bean seed halves.

?   Do any parts of the bean seed appear to have starch in them? If so, which parts?

_____

?   What structure contains stored nutrients in a mature bean seed? _____

What is the ploidy of the cells in that structure? _____

?   Did any parts of the bean seed stain pink/red with triphenyltetrazolium chloride? If so, which parts?

_____

Explain the presence of oxidation-reduction reactions in the stained parts.

Summary Comparison of Bean and Corn Seeds

The embryo of the mature corn seed constitutes about one-third of the entire seed; the remainder is endosperm. In contrast, almost the entire mature bean seed is embryo. During the development of the bean seed the endosperm is degraded and its nutrients are taken up by the cotyledons, yielding an embryo with relatively large cotyledons compared to the size of the embryonic axis.

Morphology of Young Plants (Seedlings)

In this exercise you use the seedlings which developed from the germinating seeds you planted last week.

❑   Organize yourselves into your seed planting pairs from last week, and obtain the tub you prepared.

❑   Look for plants emerging from the vermiculite.

The emerging corn shoot <u>pushes</u> straight up through the vermiculite. Its leaves and apical meristem, which originated in the **plumule**, are surrounded by the **coleoptile** which protects the growing shoot as it enlarges under the vermiculite.

The emerging bean shoot has an inverted "U" in the vermiculite. The inverted "U" is a hook in the growing stem which <u>pulls</u> the young leaves and shoot tip up through the vermiculite. The shoot's apical meristem is protected by the young leaves and cotyledons. The hook in the stem of a dicot seedling is maintained by an asymmetric distribution of ethylene gas (a plant hormone). Ethylene inhibits the growth of cells in the inner side of the hook.

❑   Each pair uses a wash pan 2/3 full with tap water in a sink for this procedure. Lay each tub with vermiculite and seedlings sideways into the water and gently loosen the vermiculite from the tub. Remove the tub and carefully wash away the vermiculite from the roots of the seedlings. Leave the water and vermiculite in the pan.

❑   Use the following procedures and questions to study the seedlings.

Each seedling consists of a developing shoot and root system which are using nutrients originally stored in the seed for growth. Bean is a "eudicot;" its two fleshy cotyledons are attached to its stem. Corn is a "monocot;" its single cotyledon remains in the seed during germination.

❑   Place each seedling in a large petri dish and observe the root system on a dissecting microscope with a black stage plate.

The root system has developed from the radicle, which you observed last week, and now consists of a main root with a few branch roots. Each branch root is similar in structure to the youngest part of the main root. Each root's apical meristem is covered by a conical mass of cells, the **root cap**, which protects the meristem from abrasion as it pushes through the soil (see Figure 8.5). Behind the apical meristem, the cells elongate and then differentiate into the mature tissues of the primary root. The presence of **root hairs** indicates the region of differentiation. A root hair is an outgrowth of the cell wall and membrane of an epidermal cell. Collectively, the root hairs greatly increase the absorptive surface area of the epidermis.

?   Can you see root hairs? They may have been removed when the seedling was separated from the vermiculite.

Bean seedlings

❑   Identify the following structures: cotyledons, primary leaves, hypocotyl, and epicotyl.

❑   In the space provided on the next page make a sketch of the bean seedling and label the parts you identified.

When a bean seed germinates, the cotyledons do not remain in the soil, but are lifted above it by the growth of the plant axis. This type of germination in which the cotyledon(s) is/are carried above the ground is called *epigeous*. The region of the plant between the root and the cotyledonary node is the **hypocotyl** (below the cotyledon). Very young seedlings usually have a strong curvature in the hypocotyl called the "hypocotyl hook." The function of the hypocotyl hook was explained in a preceding paragraph. Straightening of the hypocotyl hook in older seedlings is induced by exposure to red light, which normally would occur after emerging from the ground. The region of the plant above (beyond) the cotyledons is the **epicotyl** which develops into the plant shoot.

Corn seedlings

❑   Identify the shoot and coleoptile. Where is the cotyledon?

Seed germination in which the cotyledon(s) remain below the ground is called *hypogeous*.

❑   In the space on page 185 make a sketch of the corn seedling and label the parts you identified.

Recall from your initial observation of the corn embryo that the plumule (embryonic shoot) was encased in a sheath, the **coleoptile**. The coleoptile grows with the shoot to maintain a protective covering around the delicate shoot apex and young leaves as they push up through the soil. Notice how the leaves are rolled inside the coleoptile. Shortly after the coleoptile-encased shoot emerges from the soil, the coleoptile stops growing, but the shoot continues to grow, rupturing the tip of the coleoptile.

?   Is the germination of bean seeds epigeous or hypogeous?

SKETCHES

EFFECT OF LIGHT AND DARKNESS ON PLANT SHOOT DEVELOPMENT: A DEMONSTRATION

The seedlings you observed in the previous study are 9 or 10 days old. The seedlings for this demonstration are a few days older.

❑ Compare bean plants grown in total darkness to bean plants of the same age grown under continuous illumination.

❑ Compare dark-grown corn plants with corn plants of the same age grown in continuous light.

❑ Record the apparent differences between plants grown in light and plants grown without light in Table 8.5.

Table 8.5. Differences between light-grown and dark-grown plants of the same age.

Plant species	Characteristic for Comparison	Growth Regimen	
		24 hr light/day	24 hr darkness/day
Phaseolus vulgaris (bean)	Pigmentation (color) Relative size of leaves Cotyledons present or absent Relative length of hypocotyl (stem below cotyledon node) Hypocotyl/epicotyl hook present or absent		
Zea mays (corn)	Pigmentation (color) Relative size of leaves Relative length of coleoptile		

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BioG 1108   **Name** _____

                                                 Lab. Instructor _____

Angiosperm Growth and Development Worksheet

1.   **Comperative morphology question:** List ALL the differences between <u>monocot and eudicot</u> plants in the space below. List all the differentiating characteristics, including those you learnt during the plant diversity lab earlier this semester (BioG 1108). Very briefly describe how these characteristics differ in these two plant groups. Bullet points are accepted; this is not an essay question. Make sure your answer is complete and includes all characteristics from germination through cell structure.

2. Essay question: Write a complete and concise answer to the following question. Include only information which is relevant. Please type your answer using 12-point font and limit your answer to (maximum) one page double-spaced.

    During the germination of a seed, the root of the young plant usually becomes established before the shoot (stem and leaves, collectively). Considering the major functions of the cotyledons, the root and the shoot and the factors most limiting to the plant's early development, why is it advantageous for the root to grow and develop more rapidly than the shoot does?

CHAPTER 9 – FRESHWATER ECOLOGY I – Invertebrate diversity

LABORATORY SYNOPSIS

In this final laboratory you will be involved in a class study of the species diversity found in an aquatic ecosystem. Your class will travel to a local pond where you will employ different techniques to sample aquatic organisms. You will then return to lab and obtain experience classifying the organisms in your samples and observing some adaptations for living in a aquatic environment.

LABORATORY OBJECTIVES

At the end of this laboratory you should&

1.   understand the basic structure of aquatic ecosystems.

2.   know some field techniques for collecting biological samples in an aquatic environment.

3.   become familiar with the broad categories of aquatic organisms as well as the sampling methods used to collect them.

4.   be able to visually recognize (from preserved specimens or photographs) certain commonly encountered freshwater organisms.

5.   have observed some adaptations to life in an aquatic environment.

6.   become proficient with the use of a dichotomous key for classifying organisms.

READING ASSIGNMENTS

In this chapter (should be read before lab)
Aquatic Ecosystems to Analysis of Pond Samples . . .   p. 189-192

Biology (Campbell and Reece 2008)
Chapter 50:   Seasonality   p. 1158
                   Concepts 52.3   p. 1159-1165

Jon C. Glase

Revised, June 2010
Mark A. Sarvary

Questions to prepare you for this Laboratory

1.   In aquatic ecosystems, what are the key differences between standing water and running water habitats?

2.   Which of the three zones in a standing water habitat is probably the most productive? Why?

3.   In a running water habitat where might you find organisms that are similar to some of those found in a standing water habitat?

4.   To which classification of aquatic organisms would crayfish be placed? Why?

5.   What supplies the largest proportion of the energy used by stream organisms?

6.   In which habitat, standing water or running water, would you expect to find the greater abundance of plankton and why?

AQUATIC ECOSYSTEMS

An **ecosystem** is the community of organisms living in a discrete area interacting with the physical environment in that area. The composition and structure of biotic communities are thus influenced by, and impact upon, the physical parameters of the habitat. Environmental factors that are most important in an aquatic or freshwater ecosystem are water current, transparency (affecting solar input), temperature, O_2 content, nutrient content (particularly phosphorus and nitrogen), pH, and the substrate (bottom) composition.

The **food web** of a community determines both the path by which energy flows and the way in which chemicals cycle through the ecosystem. At the base of any food web are the autotrophs; **producers** such as plants, and photosynthetic protists. The heterotrophs include the **primary consumers** (herbivores), **secondary consumers** (carnivores and parasites), and the **decomposers** (fungi, bacteria, and detritivores). **Detritus** is the name for dead organic material and feces in a food web; and a large assemblage of animals, called **detritivores**, make their living from this abundant food source in both aquatic and terrestrial environments.

Freshwater habitats are of two basic types (Fig. 9.1). **Standing water** habitats are those characteristic of lakes, ponds and swamps, whereas **running water** habitats are dominated by flowing water (e.g., rivers and streams). While the unidirectional movement of water is the criterion for separating these two habitat types, it should be noted that large bodies of standing water such as lakes also have moving water in the form of waves and strong currents, while flowing water systems such as streams may also have pools or other still regions. Thus the key differences between standing water and running water habitats are the overall degree of water movement and the relative amount of land-water interchange.
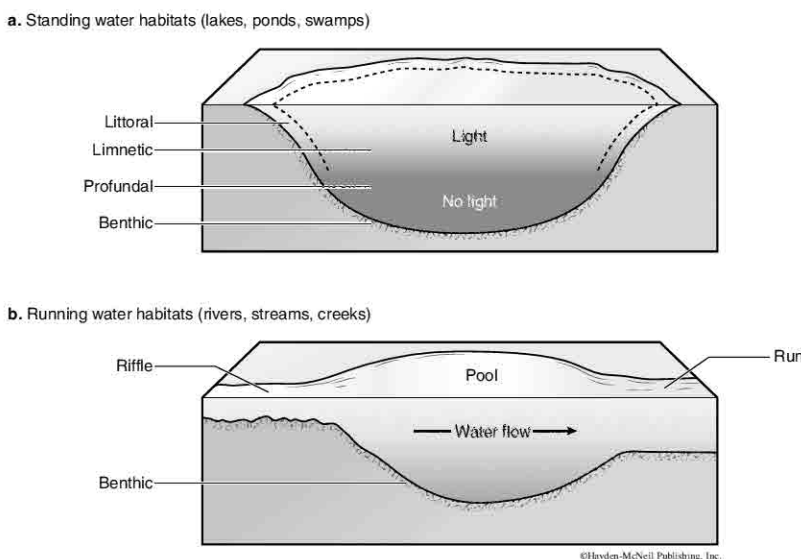
Figure 9.1. Diagrammatic representations of 1) standing water and 2) running water habitats. The arrow indicating direction of water flow applies only to the running water example.

Several regions or areas with specialized habitats are identified in standing water and running water habitats. A pond or lake may be divided into littoral, limnetic, and profundal zones. The **littoral** region is the area of shallow water near the shoreline in which rooted plants may be growing. The **limnetic** zone is the open water region to the depth of effective light penetration. The **profundal** zone is the area of deep water into which photosynthetically active light does not penetrate. The profundal zone is often absent from ponds because most of them are relatively shallow.

Streams can also be divided into three regions: riffles, pools, and runs. **Riffles** are shallow areas where the current is swift enough to keep the substrate washed clear of silt. **Pools** are deeper areas with

slow moving water where the bottom is often soft and silty. **Runs** are the deep areas of fast moving water found in large streams and rivers. As you might expect, the organisms making up the biotic community will vary among each of these habitats.

The bottom area in both standing and running water habitats is called the **benthic** zone.

THE CLASSIFICATION OF AQUATIC ORGANISMS

Aquatic organisms are often classified on the basis of their habits or locations within the environment. **Plankton** include microscopic protists (mostly algae), called **phytoplankton**, and animals, called **zooplankton**. Due to their small size, plankton may appear to remain stationary or to move about passively in the water. However, many plankton species (especially the animals) are capable of considerable vertical and horizontal movements, either on a daily or seasonal basis. Based on the method of collection, these organisms are often divided into categories of **net plankton** and **nanoplankton**. Net plankton can be caught in a fine-meshed net and consist mainly of zooplankton (e.g., small crustaceans and rotifers) and a few large phytoplankton (colonial algae). Nanoplankton are collected from water first passed through the plankton net and then filtered through a special filtering apparatus. These microscopic organisms are mainly phytoplankton (small algae and diatoms).

Periphyton are the organisms that grow attached to some underwater substrate. They include primarily algae and protists, but other organisms such as rotifers, fungi, and bacteria are sometimes placed in this category as well. They can be collected by scraping the surfaces of rocks and plants, or by providing an artificial surface which is colonized by periphyton and then collected for study.

Nekton include the organisms that swim, such as fish, amphibians, and certain large aquatic insects. **Neuston** are the organisms that live on the surface of the water, such as water striders. Both of these types of organisms can be collected with nets.

The bottom dwellers found in the benthic zone are called the **benthos**. They include organisms that burrow into the substrate, live within the crevices of the substrate, or move through the vegetation along the bottom. Various worms, mollusks, crustaceans, and insects are considered benthic organisms. They can be collected with several types of larger meshed nets (e.g., dip nets, Surber samplers) or with an apparatus designed to remove a portion of the substrate.

ADAPTATIONS FOR LIFE IN AQUATIC HABITATS

Protists, plants, and animals show a variety of adaptations for living in flowing or standing water. Below are presented a few reasons for the variations and some common organisms and the adaptations you should see on your collected specimens.

Stream Life

The ability to resist strong current flow is an important attribute for stream dwellers. As a result, the stream biota consists in large part of rooted or attached plants, as well as burrowing, clinging, or strongly swimming animals. In most streams, the constant mixing of water and the larger surface area exposed to the air result in a higher O_2 content than is typically found in ponds and lakes. This has profound effects on the design of respiratory surfaces, and as a result aquatic plants and animals have developed diverse adaptations for gas exchange in O_2-rich (stream) and O_2-poor (pool) environments.

Streams also have a larger area of shoreline than is usually found in ponds or lakes, resulting in a greater land-water interchange. A great deal of organic material enters a stream from terrestrial vegetation (such as falling leaves). The food webs of streams therefore tend to be heterotrophic and based on detritivores, with the autotrophs (attached filamentous algae and diatoms) supplying only a small proportion of the energy used by organisms in the stream.

Pond Life

Pond organisms, on the other hand, do not have to resist being washed downstream, and thus standing water habitats are characterized by an abundance of planktonic protists and animals. Ponds are much more likely to be autotrophic, with large quantities of primary production (via phytoplankton) providing the basis for the food web.

After you return to lab with the samples of living aquatic organisms collected by your class, you will have an opportunity to carefully examine their morphological adaptations for locomotion, feeding, and gas exchange. Your observations of the appearance of an organism or its activity should cause you to wonder: What does it do with a particular structure? Do the features of this structure suggest its function? How does the organism perform some life function?

METHODS FOR SAMPLING AQUATIC ORGANISMS

A study of any aquatic ecosystem requires special sampling equipment and techniques. These techniques must take into account the spatial distribution of organisms and attempt to provide an estimate of the numbers or densities of organisms within the community. Enumerating all the species in a typical ecosystem would be very difficult (and is often impossible), so ecologists usually concentrate on certain subgroups of organisms based on taxonomic affinity or common occurrence in discrete areas of the ecosystem.

Table 9.1. Sampling methods commonly used to collect aquatic organisms in the field.

Sampling Method	Category	Organisms Collected
Water sample	Nanoplankton	- solitary phytoplankton - and zooplankton
Periphyton slides	Periphyton	- diatoms and other algae - rotifers
Plankton net	Net plankton	- zooplankton - solitary and colonial phytoplankton - small crustaceans - rotifers and nematodes
Dip net	Nekton	- small fish - amphibians - swimming insects
	Neuston	- large surface swimming insects
	Benthos	- mollusks - large crustaceans - insects - worms - amphibians - fish
Minnow trap	Nekton	- small fish - amphibians - large crustaceans

STUDYING AQUATIC ECOSYSTEMS

You and your classmates will be involved in a study assessing the physical environment and the species diversity in a local pond. You should read the appropriate sections that follow.

Sampling at a Pond

Your group will sample from a single pond and compare the physical characteristics of that pond with the kinds of organisms found there.

1. Physical parameters: Water temperature is measured with a floating thermometer.

2. Benthic invertebrates: Dip nets are used to collect pond benthos. The net is dragged through vegetation and across the bottom of the pond. Large quantities of muck are to be avoided. Wash excess mud from the net and turn the net inside out into an enamel pan which is shallowly filled with water. Insect forceps and plastic pipettes are used to remove invertebrates from the sample. It is difficult to quantify replicate samples because identical dip samples are hard to take. A suggested approach is to carefully pick several of each type of organism from the sample and then note the relative numbers of each type remaining in the sample. Take at least five dip samples from the pond to be sure you have an adequate amount of material to examine upon returning to the lab.

3. Net plankton: The line from the plankton net should be stretched across the diameter of the pond. Hold the line taut, submerge the net in the water (being sure to remove any trapped air from the net as best you can), and rapidly tow it across the pond. The diameter of the net and the distance over which it is towed are used to calculate the volume of water sampled. At least three separate tows should be made to adequately sample the pond. Algae and bottom muck should be avoided. Wash down the sides of the net with a squirt bottle, and empty the collecting jar into a labeled plastic jar. Look carefully for swimming zooplankton and volvox colonies. You may remove any large insects from the sample.

Analysis of Pond Samples in the Laboratory

Each pair of students should set up two compound and two dissecting microscopes and obtain some benthic and plankton samples and periphyton slides.

Benthic Samples. Each student should use the dichotomous key for pond organisms (pages 189-194) to identify several specimens. Consult the reference collection of pond organisms available in the laboratory.

Plankton Samples. Make wet mounts of some of the plantkon samples using depression slides and coverslips. Examine at low power magnification. Use the algae and zooplankton keys and posted photographs available in the laboratory to identify the organism you observe.

Periphyton Slides. To prepare periphyton slides for examination with a compound microscope, use a safety razor blade to scrape clean one side of a slide. Add several drops of pond water to the unscraped side and carefully apply 1-2 coverslips. The slide should then be positioned on a compound microscope stage and examined at low power. Switch to high power to view the periphyton organisms you observe. Use the diatom and algae keys and posted photographs to help you identify the periphyton.

? What organisms are represented in the plankton tow samples? What kinds of adaptations do they show for their free-floating lifestyle?

Distinguishing features of certain pond insects

Some of the insects you collected are in an immature stage:

> **Larva**, if the species undergoes complete metamorphosis

> **Nymph**, if the species undergoes incomplete metamorphosis

Odonota: Damselfly and Dragonfly Nymphs

Nymphs of both groups are carnivores with an elbowed mask-like labium (lip) which can be quickly extended and retracted to capture prey.

Damselfly Nymphs

Three external gills on the end of the abdomen are used for respiration. The gills have a network of vessels to transport respiratory gases.

Dragonfly Nymphs

Pulsations of the abdomen move water into and out of a rectal respiratory chamber with internal gills. A drop of India ink placed in the water near a nymph's posterior end will enable you to see the in/out movement of water in the respiratory chamber.

Hemiptera: Water Striders

Water striders are readily identified by their ability to "walk" on water. Observer one closely and you can see that it uses its middle pair of legs to "skate" across the water surface.

The water strider possesses piercing and sucking mouth parts; it is a carnivore and generalized feeder. Its forelegs are adapted for grasping prey.

Hemiptera: Backswimmers

Backswimmers may bite; handle them with forceps. Grasp the sides of a backswimmer with flexible forceps and slowly push it about an inch below the water surface. Note how the backswimmer holds a supply of air, which looks like a silver bubble, on the underside of the body.

The boat-shaped back and paddle-like legs are adaptations for swimming.

Hemiptera: Water Boatmen

A water boatman has long hind legs flattened like oars to "row" through the water.

Hemiptera: Giant Water Bugs

Water bugs may bite, so handle them with forceps.

Water bugs obtain air at the water's surface through two short posterior siphon tubes. When gathering air the insect's body is under water with its posterior end at the water's surface.

Giant water bugs have piercing and sucking mouthparts. They are carnivorous, feeding on insects, tadpoles and small fish.

Hemiptera: Water Scorpions

The adult water scorpion is lean and long in body and appendages. It uses a pair of long tubes on its posterior end as snorkels to accomplish gas exchange at the water surface when it is under water. Observe it in an aquarium attached to plants to see its normal orientation in water - - head down, snorkels up.

Coleoptera: Whirligig Beetles

Observe a whirligig beetle swimming. Its fast movement on the water surface is achieved by the rapid paddling of its short, flattened middle and hind pairs of legs. The whirligig beetle swims with the ventral half of its body under water and the dorsal half of its body above the water surface. Its eyes are divided into two parts so that it can concurrently see above and below the water surface. It appears to have four eyes.

Coleoptera: Diving Beetles

A diving beetle carries a bubble of air to breathe when it is under water.

Coleoptera: Scavenger Beetles

A scavenger beetle traps air under its body making its underside appear silvery when it is under water. You can observe this by placing a beetle in a clear container of water.

Trichoptera: Caddis Flies

The larvae of many caddis flies are readily identified by the presence of a tubular case enclosing all but the anterior end of the organism. The larvae of pond species generally use small pieces of plant material to construct their cases. The larvae of several stream species build their cases from small stones. Some species of caddis files to not build cases.

Diptera: Mosquitoes

Mosquito larvae use a respiratory siphon like a snorkel to accomplish gas exchange at the water surface. The larvae also use brush-like structures to sweep food into their mouths.

Diptera: Bloodworms

Bloodworms are segmented worm-like organisms that are the larvae of species of midges. Their red color is due to the presence of hemoglobin-like molecules in their hemolymph used to extract oxygen from the water.

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**KEY TO THE COMMON POND INVERTEBRATES
OF NEW YORK STATE** [*]

(best used on animals > 1 mm in length)

1 a.	3 or more pairs of jointed legs	2
1 b.	Fewer than 3 pairs of jointed legs, or none	12
2 a.	More than 4 pairs of jointed legs -- Crustaceans (crayfish, sideswimmers, sowbugs, etc.) (**Crustacea**)	
2 b.	3 or 4 pairs of jointed legs	3
3 a.	4 pairs of jointed legs -- Arachnids (spiders, mites) (**Arachnida**)	
3 b.	3 pairs of jointed legs -- Insects (**Insecta**)	4
4 a.	With large functional wings, or with shell-like wings (as in beetles)	5
4 b.	Wingless, or with developing wings (wing pads)	6
5 a.	Front wings hard and shell-like; mouth not beak-like -- Beetles, adults (**Coleoptera**), see Coleoptera Key, page 364	
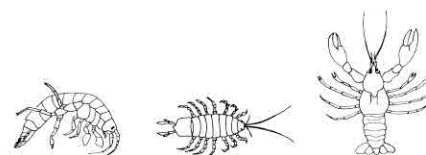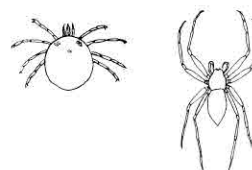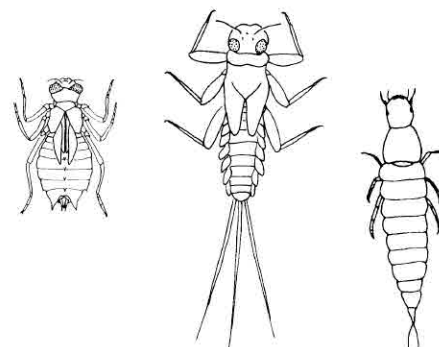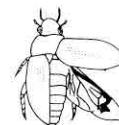
5 b.   Front wings soft or leathery; mouth
        beak-like -- True Bugs, adults
        (**Hemiptera**), see Hemiptera Key, page
        363

6 a.   With developing wings (wing pads)   7
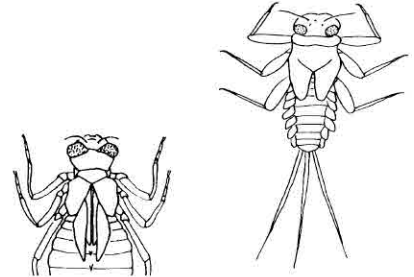
6 b.   Wingless   10
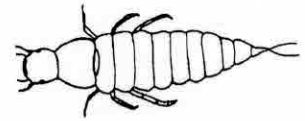
7 a.   With 2 or 3 long tail appendages   8
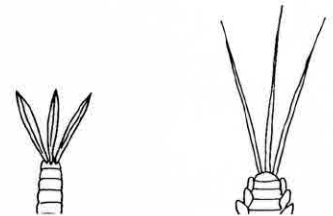
7 b.   Tail appendages very short or absent   9

8 a.   Tail appendages leaf-like and flattened;
        tail motion when swimming is side to
        side -- Damselflies, nymphs (**Odonata**)
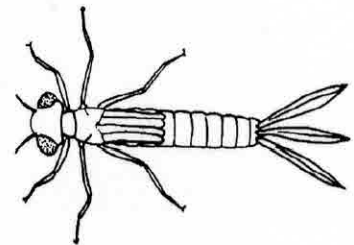
8 b.   Tail appendages thread-like; tail motion
        when swimming is up and down --
        Mayflies, nymphs (**Ephemeroptera**)
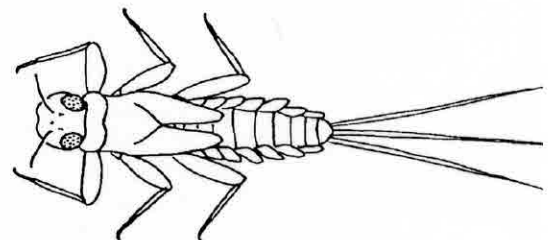
9 a.   Mouth beak-like --True Bugs, nymphs (**Hemiptera**), see Hemiptera Key, page 363

9 b.   Mouth an elbowed, spoon-like grasping organ -- Dragonflies, nymphs (**Odonata**)
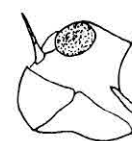
10 a.   Minute (< 1 mm); on water surface, jumping -- Springtails (**Collembola**)
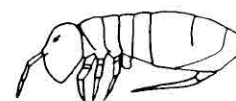
10 b.   Larger than 1 mm; underwater   11

11 a.   Usually with a case made of small stones or plant matrial; antennae nearly invisible -- Caddisflies, larvae (**Trichoptera**)

11 b.   Without a case; antennae visible -- Beetles, larvae (**Coleoptera**) and Fishflies and Alderflies, larvae (**Megaloptera**)
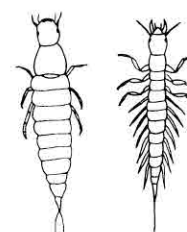
12 a.   Body with a sucker on one or both ends, wormlike -- Leeches (**Hirudinea**)

12 b.   Body without suckers   13

13 a   Body with tentacles at one end -- Hydra (**Cnidaria**)

13 b.   Body without tentacles   14

14 a.   Body with a hard shell -- Mollusks (snails and clams) (**Mollusca**)

14 b.   Body without a hard shell   15

15 a.   Body flat; animal glides along bottom -- Flatworms (**Platyhelminthes**)

15 b.   Body tubular; animal does not glide along bottom   16

16 a.   Body extremely elongate, extensible --
         Aquatic Earthworms (**Oligochaeta**)
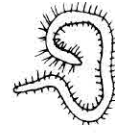
16 b.   Body not extremely elongate, rarely
         extensible -- Flies, larvae (**Diptera**)
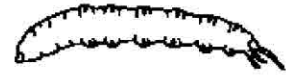
KEY TO HEMIPTERA ADULTS

1 a.   With 2 long tail appendages -- Water Scorpions (**Nepidae**)
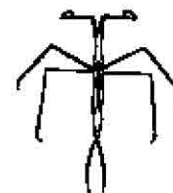
1 b.   Tail appendages short or absent   2

2 a.   Wings and/or area in front of wings nearly white; swim belly-up -- Backswimmers (**Notonectidae**)

2 b.   Back dark or patterned, never white   3

3 a.   Very small (< 3 mm); swim belly-up -- Pygmy Backswimmers (**Pleidae**)

3 b.   > 3 mm; swim belly-down   4

4 a.   Large, > 18 mm -- Giant Water Bugs (**Belostomatidae**)
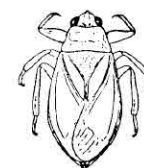
4 b.   Smaller, < 18 mm   5

5 a.   Extremely thin and delicate; inhabits water surface (neuston)-- Water Measurers (**Hydrometridae**)
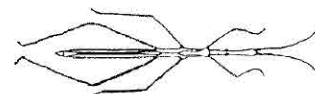
5 b.   Not so thin and delicate   6

6 a.   Marbled pattern on wings; very short beak -- Water Boatmen (**Corixidae**)
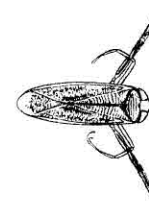
6 b.   No marbled pattern on wings; beak at least longer than wide   7

7 a.   Front legs extremely thickened -- Creeping Water Bugs (**Naucoridae**)
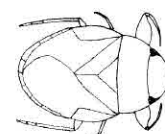
7 b.   Front legs not thickened; inhabits water
          surface (neuston) -- Water Striders
          (**Gerridae, Veliidae, Mesoveliidae**)
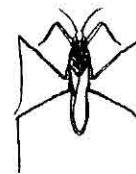
KEY TO COLEOPTERA ADULTS

1 a.   Two pairs of eyes; two hind pairs of
          legs short and extremely flattened;
          usually on water surface -- Whirligig
          Beetles (**Gyrinidae**)
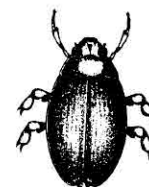
1 b.   One pair of eyes; hind pairs of legs
          long, not extremely flattened   2

2 a.   Head very small, not in a smooth
          outline with the rest of the body; body
          usually with yellow and black pattern,
          2.5 to 5 mm -- Crawling Water Beetles
          (**Haliplidae**)
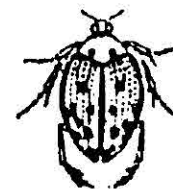
2 b.   Head larger, making a smooth outline
          with rest of body; pattern and size   3
          variable

3 a.   Underside not silvery when in water
          (living beetles only); hind legs stroke
          backward together -- Diving Beetles
          (**Dytiscidae**)
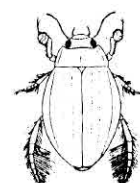
3 b.   Underside silvery when in water (living
          beetles only); hind legs stroke
          backward alternately -- Water
          Scavenger Beetles (**Hydrophilidae**)
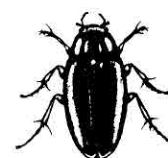
Notes:

Chapter 10– FRESHWATER ECOLOGY II: water quality assessment and nutrient enrichment

**Kuei-Chiu Chen, Margery G. Spofford, Jerry A. Waldvogel,
Martha R. Taylor, Jon C. Glase, Paul R. Ecklund and Mark Sarvary**

LABORATORY SYNOPSIS

In this laboratory you will be engaged in a multi-week project of scientific investigation that allows you to design your own experiment on nutrient enrichment and present your data in a lab report. The sequence of activities varies based on the season. The first weeks will be an in-lab nutrient enrichment experiment for algal growth using water collected from the pond or from a freshwater source chosen by your group. Your group may also chooses a nutrient or a combination of nutrients of interest and designs an experiment to produce data for statistical testing. As chlorophyll-a concentration is a good indicator of biomass of phytoplankton in aquatic systems, you will use a few standard analytical techniques to calculate chlorophyll-a concentration to estimate algal biomass. Your study group will use this research process to develop a lab report at the end of the semester.

LABORATORY OBJECTIVES

Conceptual

At the end of this laboratory you should&

1. understand the basic structure of aquatic ecosystems.

2. have observed and be able to interpret how a certain morphological feature has adapted to life in an aquatic environment.

3. understand the limiting nutrient and its connection to eutrophication.

4. understand why chlorophyll-a is use as an indicator for algal biomass estimation.

5. be able to evaluate data by choosing appropriate statistical tests mentioned in this lab.

READING ASSIGNMENTS

In the lab manual:

Day One

Introduction to Day one

Day Two

Introduction to Day two

For additional information see pages 1231-1242 in *Biology* (Campbell et al., 2008).

Questions to prepare you for this Laboratory

1. In aquatic ecosystems, what are the key differences between standing water and running water habitats?

2. Which of the three zones in a standing water habitat is probably the most productive? Why?

3. What is a limiting nutrient in an aquatic system? What is eutrophication and what causes it?

4. What supplies the largest proportion of the energy used by stream organisms?

5. In which habitat, standing water or running water, would you expect to find the greater abundance of plankton and why?

INTRODUCTION TO DAY ONE – NUTRIENT ENRICHMENT

As described in the Nutrient Content in Aquatic Systems section, algae are able to utilize their limiting nutrient(s) when it is available and reproduce rapidly to create algal bloom. Although algae are photosynthetic thus are able to produce oxygen during the day, their high density drastically reduces dissolved oxygen level at night when they respire but not photosynthesize. This causes extreme fluctuation of dissolved oxygen concentration in a daily cycle when algae concentration is high. Furthermore, when the nutrients are depleted and the algae die off en masse, bacteria soon decompose algae for food and consume dissolved oxygen in their respiration process. The oxygen depletion then causes deaths in fish or reduces their reproductive potential (see Campbell et al. 2008, pg. 1231-1242). The underwater barrens of low fish density and other larger aquatic animals near the mouths of rivers in oceans or lakes are known as the dead zones, which are often caused by fertilizers in agricultural regions that leach into these rivers which in turn cause algal bloom and death in oceans or lakes.

In this experiment you may choose either a chemical that contains a specific element or a category of substances such as a fertilizer that potentially contains the limiting nutrient for algal growth in the freshwater you use.

Research hypothesis: If your chosen substance indeed contains the limiting nutrient for the algae, it should increase the biomass of algae at the end of the experimental period.

PROCEDURES FOR DAY ONE – SET UP THE EXPERIMENT

The experiment is designed to simulate a continuous release of nutrients into the freshwater bodies as in the natural leaching process of nutrients in the soil that to streams, lakes or oceans.
Each group is provided with the following materials:

    6 – 12   2" terra cotta pots (30 ml capacity)
    6 – 12   500 ml wide-mouth jar with lid
    2% melted agar in water bath
    Parafilm
    Stir stick
    Petri dish

Each group may choose to use the following provided items for testing:

    Miracle-gro® All Purpose Plant Food
    Osmocote Smart-Release® Plant Food

OR you can bring your own nutrients to test.

If you choose to bring your own potential nutrients, you will need to provide a small amount of other fertilizer detergent, vitamins, OTC pain medicine, etc. **These nutrients/chemicals must be approved by your lab instructor a few days before the lab.**

Pond water will be provided by your lab instructor.

1. Apply a small piece of masking tape to cover the drain hole of each plant pot from inside. Press the tape with the eraser end of a pencil or a similar item to seal. Label side of each pot with a masking tape indicating either control or experimental group, the replicate number, and your group name.

2. Pour 2% liquid agar to each plant pot about half full, add desired amount of nutrient and gently stir to mix well. Immediately add more agar until the pot is almost full. Let agar solidify without disturbance.

3. After the agar is solidified, seal the large opening of the pot by placing a piece of Parafilm to cover the opening and run your finger around the rim. Place a small petri dish over the parafilm and secure it with a rubber band. Fold back the extra edge of Parafilm to allow water to fully contact the pot surface (see below).

Sealing the large opening of plant pots with Parafilm and the petri dish minimizes rapid release of nutrients through this opening.

4. Remove label from the assembled pot and place it on a 500-ml jar. Invert the pot and gently place it into the jar receiving the label. Add 250 ml of freshwater to allow complete submersion. Place lid on jar without screwing it tight to allow gas exchange.

5. Repeat this procedure to all replicates in experimental group.

6. Repeat procedure to all replicates in control group except adding nutrients.

7. Leave jars undisturbed at designated location until next week.

INTRODUCITON TO WEEK TWO – CONCENTRATION OF CHLOROPHYLL-A

Photosynthesis is a process for organisms to transform light energy to chemical energy using specific proteins that absorb light at specific wavelengths. At the core of these reactions are many pigment molecules that absorb certain wavelength(s) of light and emit or reflect other wavelengths of light in the visible spectrum between 400 nm to 700 nm. Although the composition of pigments vary based the taxonomy of the photosynthetic organisms, a great majority of photosynthetic organisms require chlorophyll-a to covert light energy to chemical energy, so the concentration of chlorophyll-a is a good indication of the mount of primary production.

Chlorophyll-a has two absorption peaks, one at the blue color range around 430 nm and the other at the red range of 665 nm. Because the wavelengths within the green color range are reflected, we see chlorophyll-a as a green pigment. In its pure form, chlorophyll-a concentration can be measured at either one of its two absorption peaks. However, because many accessory pigments also absorb light at the blue color range in a natural extract from algae or other photosynthetic organisms, we should use 665 nm as the wavelength in spectrophotometry for chlorophyll-a concentration (Figure 10.2).

The structure of chlorophyll-a is made up of a ring of carbon and nitrogen with magnesium at the center and a long chain of mostly hydrocarbon. When zooplankton consume algae, the acidity in their digestive tracts convert chlorophyll-a to various types of pheopigments, for example, as pheophytin-a by losing the magnesium from the center of its ring structure or as pheophorbide-a by losing both its

magnesium and its hydrocarbon chain (Figure 10.3). These pheopigments have similar absorption peaks, but at a weaker absorption level. Because these pheopigments are not photosynthetically active, they do not contribute to the primary production of algae, so the reading from these pigments should be subtracted from the total reading of chlorophyll-a.
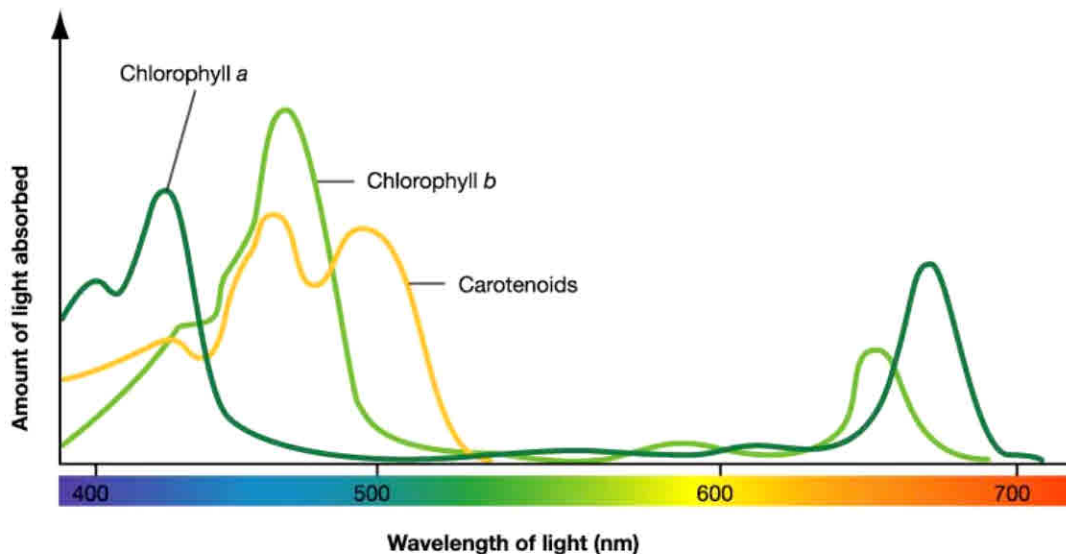
Figure 10.2. Absorption spectrum of chlorophyll-a, chlorophyll-b, and carotinoids. Notice that the color of pigments are from the wavelengths of light that are NOT absorbed.
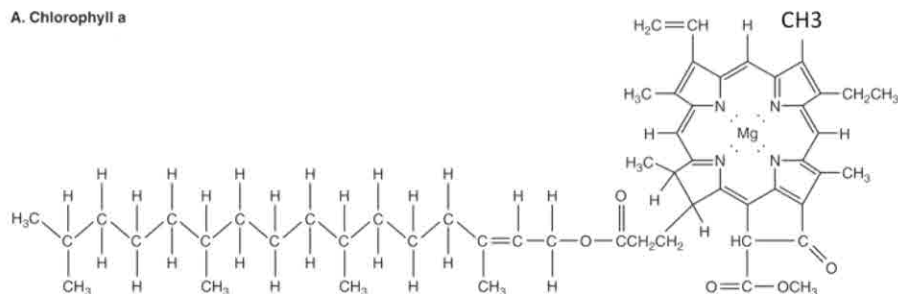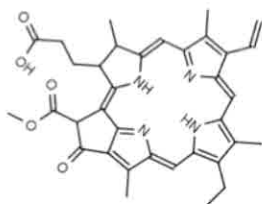
Figure 10.3. Structure of chlorophyll-a and two of the common pheopigments, pheophytin-a and pheophorbide-a.

PROCEDURES FOR DAY TWO – CHLOROPHYLL-A CONCENTRATION

Today you will use the concentration of chlorophyll-a as a measurement to estimate the level of primary production from algae in freshwater. You will first use a water pump to concentrate algae from a fixed volume of water and collect algae on a cellulose filter. You will then extract chlorophyll-a and other photosynthetic pigments from the filter with 95% ethanol placed in a hot water bath. The ethanol extract will be transferred to a cuvette and the percent transmittance of light at 665 nm is measured using a spectrophotometer. As described in the introduction for today's activity, the presence of pheopigments can overestimate the presence of chlorophyll-a. After completing the initial reading spectrophotometric reading, you will acidify the pigment extract to turn all chlorophyll-a to pheopigments and conduct the reading of percent transmittance again. You will then convert the percentage transmittance to absorbance using Table 1 from Appendix – Instrumentation (BioG 1107). Using the provided formula, you will estimate the concentration of chlorophyll-a as a measure of productivity.

Material list per group

1	test tube brush or hard bristle toothbrush
1	test tube holder
1	forceps
1	small scissors
1	10 ml graduated cylinder
12	Test tubes
1	bottle of 95% ethanol
1	5 ml glass pipette with dispenser
12	cuvettes
1	strip of aluminum foil
1	strip of Parafilm®

Filtration Stations

There are 3 filtration stations, each has

1	filtration device (made up of ground glass base, rubber stopper, 2-L vacuum flask, funnel, clamp, supporting stand and aspirator tubing connected to faucet)
1	box of cellulose filters
1	100 ml graduated cylinder

Scrub algae from plant pot and walls of jar

↓

Filter algae from water; pat dry filter

↓

Cut algae-containing filter and add 10 ml 95% ethanol

↓

75°C water bath for 5 min.

↓

Transfer 5 ml pigmented ethanol to cuvette

↓

Spec 20 reading for Chlorophyll a on % Transmittance; record data

↓

Add 150 µl of 0.1N HCl; invert cuvettes twice to mix; let stand for 1.5 min.

↓

Spec 20 reading for phaeopigments on % Transmittance; record data

↓

Repeat procedures for other samples if necessary

↓

Convert Transmittance to Absorbance using Appendix table

Figure 10.4. Experimental flow chart for filtering algae, ethanol extraction and spectrophotometer reading.

Collection of algae

For all sample jars:

1. While plant pot is in the jar use test tube brush to thoroughly brush off algae attached on surfaces of the plant pot and glass jar into water.

2. Use the test tube holder or blunt probe to lift up plant pot. Discard agar and Parafilm. Place pots in washbasin next to the sink.

3. With the test tube brush do a final removal of algae from the inside of the jar; clean and blot dry brush between samples; repeat procedure for all sample jars.

4. Label each test tube. Label one extra test tube "filter blank". Bring jars, lids, scissors and labeled test tubes to filtration station.

Algal Filtration

1. Use forceps to pick up one cellulose filter and place it at center of funnel stem. Place reservoir small side down to cover filter and align with funnel. Hold parts together with clamp.

2. For each sample, tighten lid on jar and thoroughly mix algae by inverting jar 2-3 times.

3. Immediately measure 50 ml of water sample with graduated cylinder then transfer it into reservoir. **(Note: if sample water appears dense with algae, use 50 ml or less instead. Record your volume)**

4. Turn on aspirator faucet.

When water travels from a pipe of wider diameter to one with a narrower diameter, it produces a lower pressure at the pipe with narrower diameter. Since the narrower tube is connected to the side arm of the flask, the low pressure in the flask pulls water through the filter to facilitate filtration.

5. Turn off faucet and release vacuum after sample water has completely passed through filter and the filter appears dry.

6. Unclamp funnel and glass base and remove funnel. Fold filter in half with forceps, algae side in, and blot dry on paper towel.

7. Cut filter into four wedges and place them in appropriate test tube.

8. Measure 100 ml of deionized water and follow the procedure above to prepare a filter blank.

Water Bath Incubation

1. To each test tube add 10 ml of 95% ethanol. Cover each test tube with aluminum foil and incubate in 75°C water bath for 5 min.

2. While algal filters are in hot water bath, label cuvettes near their top with masking tape.

3. Retrieve test tubes from water bath. **CAUTION: the test tubes are hot; hold them by their top and bring them to your bench to let cool for 1-2 min.**

4. Mix the test tube content while holding the Parafilm on top and then transfer 5 ml of pigment extract to each corresponding cuvette and seal with Parafilm.

Spectrophotometer Reading Before And After Acidification

You should have read the Appendix on Spectrophotometry using Spectronics 20®. Your TA will show you the Spec20 video to ensure proper handling of this instrument. For measuring chlorophyll-a concentration, the wavelength should be set for 665 nm.

1. While no cuvette is in sample holder, turn zero control knob of Spec 20 to 0 %T (transmittance).

2. Insert cuvette of filter blank completely in sample holder with its white, vertical line aligning with the raised line on edge of sample holder. Turn 100%T knob to 100%. Remove blank.

3. Insert sample in sample holder in the same manner as you did with filter blank. Read %T value to the nearest 0.25%. Record data in Table 10.3

4. Repeat procedure to all prepared samples and record your data under Before Acidification in Table 2.3.

5. Add 150 μl of 0.1 N HCl to cuvette, cover with Parafilm, invert twice to mix well, and let stand for 1.5 min.

6. Measure %T after 1.5 min. Record data in Table 10.3 under "After Acidification".

7. Repeat acidification procedure to all samples and record %T.

8. Repeat Algal Filtration procedure on samples in the experimental group if necessary.

9. Use Table 1 in Instrumentation Appendix to convert %T to Absorbance and record in Table 2.

10. Subtract absorbance value after acidification from absorbance before acidification. Record data in Table 10. 3

Table 10.3. Percentage transmittance and absorbance before and after acidification.

Replicates		Before Acidification		After Acidification		Difference in Absorbance
		%T	Absorbance (A_o)	%T	Absorbance (A_a)	$A_o - A_a$
Controls	1					
	2					
	3					
	4					
	5					
	6					
Experiments	1					
	2					
	3					
	4					
	5					
	6					

Calculation of chlorophyll-a concentration

As described above, among all photosynthetic pigments chlorophyll-a is the only pigment at the center of reaction of photosynthesis in most organisms, so its concentration can provide direct estimation of the level of primary production. There is a direct relationship between the absorbance and the amount of chlorophyll and the absorbance can be converted into chlorophyll-a concentration using the following equation.

$$Chl\ a\ (\mu g/L) = \frac{29.6\ (A_o - A_a)\ x\ v}{V_f\ x\ l}$$

where

29.6 is the combination of chlorophyll-a absorption coefficient and the factor to equate the reduction in absorbancy to initial chlorophyll concentration,
A_o = Original absorbance at 665 nm,
A_a = Absorbance after acidification at 665 nm,
v = Ethanol extract volume in ml,
V_f = Volume of water filtered in liter,
l = length of light path in cuvette in cm, ours is 1.2 cm.

Calculate the concentration of chlorophyll of each of the replicate and record values in Table 10.4.

Table 10.4. Concentration of chlorophyll (μg/L) in control and experimental groups.

Replicate	Control group	Experimental group
1		
2		
3		
4		
5		
6		

After you have completed calculating the concentration of chlorophyll-a of all replicates, you should read the appropriate section in the statistics appendix to decide on appropriate statistical test. As you may quickly realize, you are conducting the experiment on two groups of data, one with and one without the treatment, in hope to see if any difference exists between them. It becomes obvious from the chart that you are making a choice between **_t_ test** or **rank sum test**. If none of your replicates have failed due to contamination or other factors, and you have a large enough combined sample sizes, such as 12, you may choose to use _t_ test because it is more powerful in detecting differences. If, however, some of your replicates turned out to be unusable, you may still conduct a statistical test using the rank sum test, which can accommodate replicate as low as a total of 8 of combined number of replicates.

t Test Procedure

As a convention on statistical tests, you always start by stating your null hypothesis (H_O) and alternative hypothesis (H_A). For our purposes, the two competing hypotheses maybe be stated as follows:

H_O = There is no difference in the chlorophyll-a concentration between control and experiment groups

H_A = There is a difference in the chlorophyll-a concentration between control and experiment groups

Consult the symbols and equations in the statistical reference appendix section on Mean, Variance, and Standard Deviation. Continue with Table 10.4 above and organize your flow of calculation as follows:

Control group	Experimental group
n_1 =	n_2 =
$\overline{X}_1$ =	$\overline{X}_2$ =
SS_1=	SS_2 =
s^2 =	
s =	
t =	

Where

$$s^2 = \frac{SS_1 + SS_2}{(n_1 - 1) + (n_2 - 1)}$$

$$s = \sqrt{\frac{s^2}{n_1} + \frac{s^2}{n_2}}$$

$$t = \frac{\overline{x}_1 - \overline{x}_2}{s}$$

You should use the sum of (n_1-1) and (n_2-1) as your total sample size and look up the tabular statistic of that sample size at the 0.05 level. If your *t* statistic is greater than the tabular statistic, you should reject your null hypothesis that the two groups have no difference. The conclusion may be stated that the two groups are significantly different in chlorophyll-a concentration. If your *t* statistic is greater than the tabular statistic at the 0.01 level, you should further conclude that the difference of chlorophyll-a concentration between the two groups of data is **highly** significant.

Rank Sum (Mann-Whitney) Test Procedure

You can use the non-parametric method of rank sum test if your sample size is smaller. As described in Appendix 3, rank sum test convert data into ranks and generate statistic. As a non-parametric method, rank sum test does not assume the distribution of sample and does not measure mean or standard deviation of samples. As always in a statistical test, you should state your null hypothesis and alternative hypothesis.

H_O = There is no difference in the chlorophyll-a concentration between control and experiment groups

H_A = There is a difference in the chlorophyll-a concentration between control and experiment groups

Follow the procedure in the Appendix C to complete the following table.

| Rank of control | Chlorophyll-a concentration ($\mu g/L$) | | Rank of experiment |
	Control	Experiment	
$T_1=$			$T_2=$
$n_1=$			$n_2=$
Test statistic (the smaller of T_1 and T_2) =			
Tabular statistic =			

If your test statistic is **smaller** than the tabular statistic at the alpha level of 0.05, you should **reject** the null hypothesis and conclude that there is a difference between the two groups of data. Again, if you can reject the null hypothesis at the alpha level of 0.01, you should conclude that the difference is highly significant.

WRITING ASSIGNMENT:

By the end of the semester (August 4, 2010) your group will need to turn in a well-organized and concise lab report of your study. The lab report should include an introduction, materials and methods, results, discussion, references and an appendix with your statistical test. Use 12-point type fonts, double spaced with one inch margins. Attempt to limit your paper to 4 pages maximum (including appendix).

REFERENCES AND SUGGESTED READINGS

Bennett, G. W. (1983). *Management of lakes and ponds* (2nd ed.). New York: Krieger Publishing Co.

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APPENDIX 1
MICROPIPETTORS

DIGITAL MICROPIPETTORS

The digital micropipettor is a precision instrument designed to fill a disposable pipette tip with a user-selected volume of solution. Different models of micropipettors allow you to "dial up" in micrometers (μl) the volume you wish to pipette within the volume range that the model provides. You will be using two models of micropipettor in this course: a 2-20 μl micropipette and a 20–200 μl micropipette.

Recall that there are 10^3 μl is a milliliter (ml) and 10^6 μl in a liter (l). Complete the following conversions:

1 μl = _____ ml   100 μl = _____ ml   0.001 l = _____ μl

Principle of Operation

A typical digital micropipettor consists of a barrel that is grasped in one hand, a shaft on to which disposable pipette tips are mounted, a knob for adjusting the volume being transferred, a plunger for filling and emptying the tip, and a tip ejector mechanism. After setting the volume adjuster to the correct volume and inserting a disposable pipette tip onto the micropipettor, the user depresses the plunger on the top of the barrel to the first, "calibrated stroke" position. The plunger pushes a piston into a cylinder a distance that is determined by the volume setting. When the plunger is released, the piston is pulled out of the cylinder and a defined volume of fluid is drawn into the pipette tip. The plunger is than depressed to the first position and on to a second, "blow out" position that dispenses the fluid out of the tip. If appropriate, the tip can either be reused, or ejected from the micropipettor. Most micropipettors are designed for one hand operation.

Pipetting Instructions

The following instructions are for the Oxford® *BenchMate* micropipettor (Figure 1):

Figure 1. The Oxford® *BenchMate* digital micropipettor.

1. *Set Volume.* First loosen the lock nut mechanism at the base of the plunger by turning it counterclockwise (Figure 2 left). Now turn the thumb screw at the end of the plunger clockwise to decrease the volume setting and counterclockwise to increase the volume setting. Be sure you understand how to read the digital display (Figure 2 right). The white horizontal line on the display represents the decimal point. DO NOT attempt to rotate the volume adjuster beyond the upper or lower limits of the pipette's range, as stated on the top of the barrel. When you have properly set the volume, turn the thumb screw to lock the instrument at that volume.
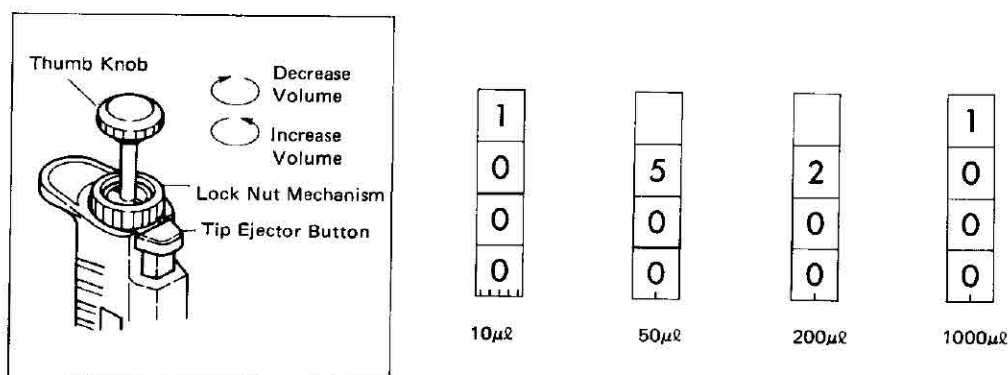
Figure 2. Left: mechanism for setting volume. Right: digital scales for various models.

2.   *Fill the tip.* Firmly insert a new tip on to the stem. Before inserting the tip into the solution, use your thumb to depress the plunger to the first stop (Figure 3). Now, insert the pipette tip into the solution to a depth of not more than 3 mm. Smoothly return the plunger to the release position, drawing liquid into the tip. Withdraw the tip from the container.
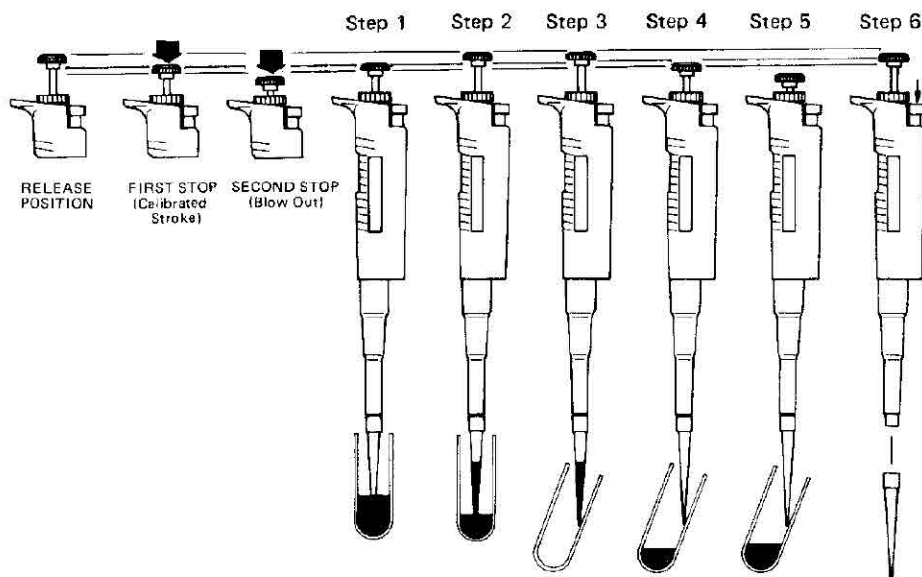
Figure 3. Filling and expelling a sample from the digital micropipettor. See the text for a detailed description of the procedure to use.

3.   *Expell the Sample.* Place the pipette tip against the side wall of the container to receive the sample. Smoothly depress the plunger to the first stop. Pause briefly, and continue to depress the plunger to the second stop. With the plunger still depress, slide the tip out of the container, leaving the sample on the side wall near the bottom.

4.   *Eject the tip.* Gently, return the knob to the release position. Do not allow the plunger to "snap" back. Remove the disposable tip by firmly depressing the tip ejector button.

The following techniques aid in pipetting accuracy and reproducibility.

a.   Smooth, consistent depression and release of the plunger insures an even intake and release of liquid from sample to sample. Never allow the plunger to "snap" back.

b.   To avoid bubble formation in the tip, always depress the plunger to the "calibrated fill" position before inserting the tip into the sample.

c.   Insert the tip to about the same depth in all samples (about 3 mm). Hold the instrument as vertically as possible as you fill the tip.

Precautions

The digital micropipettor is an expensive, precision instrument. Please follow these precautions in use of this piece of equipment.

1.   Never attempt to rotate the adjuster knob beyond the upper or lower limits for the pipettor's range.

2.   Never attempt to fill the micropipettor without a tip in place.

3.   Never allow the plunger to snap back when withdrawing or ejecting a sample.

4.   Never lay down the pipettor with a filled tip.

5.   Never immerse the barrel of the pipettor into liquid.

Pipetting Exercises

In these exercises you transfer three aliquots of different volumes into a container. You then set the micropipettor to withdraw a sample that is equal to the total volume of liquid that you added to the container. If your pipetting is accurate, the sample should just fill the pipette tip without a bubble and with no sample left over. Each exercise uses a different pipetting model. You will be pipetting distilled water to which food coloring has been added.

<u>Small</u> <u>Volume</u>—use the 2–20 μl model.

Adjust the micropipettor to add 2 μl, 5 μl, and 10 μl to a tube. You will need to "pool" the sample by briefly spinning the tube in a balanced micro-centrifuge. Set the micropipettor to 17 μl and withdraw the sample from the tube.

<u>Large</u> <u>Volume</u>—use the 20–200 μl model.

Adjust the micropipettor to add 20 μl, 50 μl, and 100 μl to a tube. Set the micropipettor to 170 μl and withdraw the sample from the tube.

An example: BIOG 1108 SUMMER LABORATORY PRACTICAL

Name: _____   TA's Name: _____

Date: _____

I HEREBY STATE THAT I WILL NEITHER GIVE NOR RECEIVE INFORMATION DURING THIS EXAM (EXCEPT THAT DELIVERED BY THE LAB INSTRUCTOR), NOR WILL I PASS ON INFORMATION ABOUT THE EXAM TO THOSE STUDENTS WHO HAVE NOT YET TAKEN IT.

Signed _____

Instructions:

1. Restrict your answers to the questions to the provided space. Answers written in margins will not be graded. Space is provided for calculations, where necessary.

2. Be precise and concise in responding to the questions. Points will be lost for "data dumping!"

3. Good luck!!!

_____

8_____ **1**. Your instructor will project several slides of different types of animals studied this semester. For each

slide, give the name of the organism, the name of the phylum, and select an appropriate term for the taxon from <u>each of the four sets</u> of terms below. No credit will be given for correct descriptive terms applied to incorrect taxa.

Type of Coelom: (a) coelomate; (b) pseudocoelomate; (c) aceolomate

Symmetry: (d) asymmetry; (e) radial symmetry; (f) bilateral symmetry

Digestive system: (g) none; (h) gastrovascular cavity; (i) tubular

Circulatory system: (j) none; (k) open; (l) closed

Slide	Common Name	Phylum	Letters of Four Descriptive Terms
1			
2			
3			
4			

7_____ **2**. Go to station 2. Using the dichotomous key provided, identify the specimen to the level of **Order**. List the numbers of the choices from the key that you selected in identifying this specimen.

Choices:_____

Order: _____

A.   Select a morphological adaptation that this organisms shows for life in an aquatic habitat and explain how it is adaptive.

| |
| |
| |
| |
| |

5_____ **3**. Go to station 3. Examine the two labeled photographs associated with this station as you answer the following questions.

A.   In the photos associated with this station, identify the numbered structures and briefly describe the function of each structure.

**STRUCTURE**   **FUNCTION**

___   _____   _____

___   _____   _____

___   _____   _____

___   _____   _____

B.   Identify the structure pointed to in the microscope associated with this question. What is its function?

Structure: Function:

10_____ **4.** Complete the following table by entering a "**+**" if the condition is true, a "**–**" if the condition is false, and **NA** if the condition is not applicable for the cell that is dividing.

Dividing Cell is…	Diploid		Haploid	
	Double Chromatid Chromosomes	Homologous Pairs	Double Chromatid Chromosomes	Homologous Pairs
Mitosis Prophase				
Mitosis Telophase				
Meiosis Prophase I				
Meiosis Prophase II				
Meiosis Telophase II				

5_____**5.** Go to station 5. Examine the dissected specimen associated with this station as you answer the following questions.

     A. What is the sex of this specimen? _____

     B. Identify the structures indicated by the numbered pins and briefly describe the function of each structure.

#	STRUCTURE	FUNCTION
1	_____	_____
2	_____	_____
3	_____	_____
4	_____	_____

5_____**6.** Go to station 6. Using the materials available, move as accurately as possible 36.5 µl of the colored dye solution into an unlabeled microcentrifuge tube. When finished, properly dispose of your tip and reset the pipettor to 20 µl. Close your microcentrifuge tube and use the marking pen to label the tube with your initials and give it to your instructor for grading.

5_____**7.** Go to station 7. Examine the dissected specimen associated with this station as you answer the following questions.

     A. Briefly describe one way in which the circulatory system of this animal is like your own circulatory system.

     B. Identify the structures indicated by the numbered pins and briefly describe the function of each structure.

#	STRUCTURE	FUNCTION
1	_____	_____
2	_____	_____
3	_____	_____
4	_____	_____

5_____**8**. Go to station 8. Examine the dissected specimen associated with this station as you answer the following questions.

    A. What structure in this animal is the functional equivalent of the funnel in the squid? _____

    B. Identify the structures indicated by the numbered pins and briefly describe the function of each structure.

#	STRUCTURE	FUNCTION
1	_____	_____
2	_____	_____
3	_____	_____
4	_____	_____

5_____**9**. A certain species of bacteria uses the biochemical pathway shown below to make a purple pigment. Two different mutant strains of the bacteria are unable to make the pigment when they are grown separately on nutrient agar. Each strain has only one step (reaction) blocked as shown on the diagram.
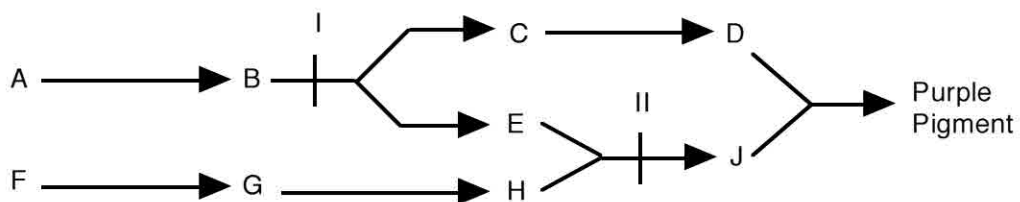
    A. When strains I and II are grown side-by-side, but not touching each other, in a feeding trial, which of the following is a possible result? (Circle the letter of the correct answer.)

    a) Only strain I will be purple.

    b) Only strain I I will be purple.

    c) Both strains will be purple.

    d) Neither strain will be purple.

    B. Briefly explain your answer.

8_____**10.** The data table gives the results of feeding trials using pairs of mutant strains of a bacterial species grown on a nutrient agar medium. Each mutant strain alone is unable to make the end product of a biosynthetic pathway because it cannot perform one of the reactions.

Strain Fed (+) or Not Fed (-)

		1	2	3	4	5	6
	1		-	-	-	-	-
	2	+		+	-	-	+
Potential	3	+	-		-	-	+
Feeder	4	+	+	+		-	+
Strain	5	+	+	+	+		+
	6	+	+	+	+	-	

A. Which strain cannot feed any other strain (if none, answer none)? _____

B. Which strain is not fed by any other strain? _____

C. Which pairs of strains show mutual feeding? _____

D. Draw a biochemical pathway that corresponds to the data presented in the data set.

11_____**11.** A. The figure below shows the sequence of events in the fertilization of an urchin egg. Items involved in the process are indicated by letters. Fill in the table – if the letter is given, write the name pf the structure, if a name/description is given, write the appropriate letter.

1.	____	Acrosomal enzymes that digest jelly coat	8.	_I_	_____
2.	_G_	_____	9.	____	Egg plasma membrane
3.	____	Acrosome	10.	____	Fertilization membrane
4.	____	Actin filaments	11.	_K_	_____
5.	_A_	_____	12.	____	Perivitelline space
6.	____	Contents of cortical granule	13.	_L_	_____
7.	____	Cortical granule	14.	____	Vitelline layer
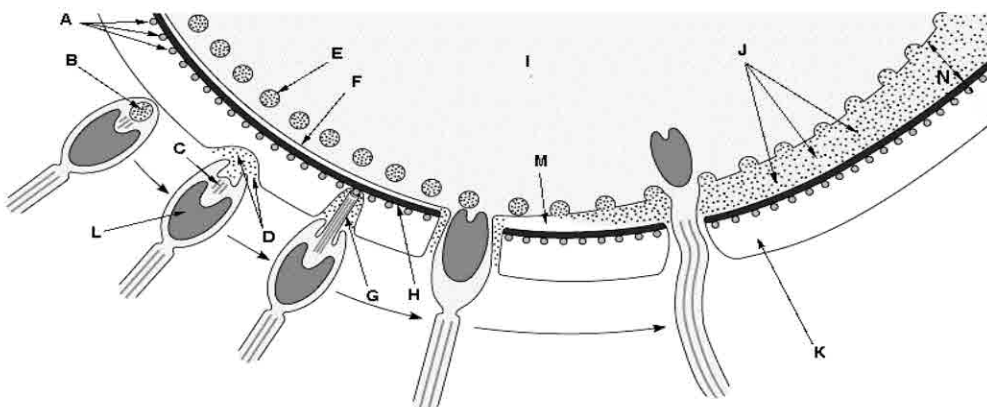
B. 1. What specific mechanism prevents other species' sperm from fertilizing the egg of a certain species of urchin?

| |
| |

   2. Why would this mechanism be particularly important to the urchin in reproduction in contrast to reproduction in the crayfish?

| |
| |
| |
| |
| |
| |
| |

16 **12.** A researcher received two colorless spore mutant strains of *Sordaria fimicola*. Neither strain could produce the black spore pigment melanin. She knew that a mutant form of only <u>one</u> gene was responsible for the absence of melanin in either strain, but she did not know whether both strains had the same mutant gene. (Note that this is different from the situation you studied in *S. fimicola*.) To determine whether the two strains had mutant alleles for the same gene or for different genes, she crossed each mutant with wild type *S. fimicola*. Below are the results obtained for 1000 asci examined from one of those crosses.

Spore Arrangement in Ascus	Number Asci Counted
●●●●○○○○	__284__
○○○○●●●●	__316__
●●○○●●○○	__82__
●●○○○○●●	__104__
○○●●●●○○	__96__
○○●●○○●●	__118__

A. What is the observed frequency of crossing over for this gene? Show your calculations.

| Frequency of crossing over = _____ |

B.   What is the map unit distance between the colorless spore gene and the centromere of the chromosome? Show your calculations.

> Map Unit distance = _____

The results of the cross between the other colorless spore mutant and wild type *S. fimicola* indicated that <u>both</u> colorless spore mutants had the <u>same</u> map unit distance between the mutant gene and the centromere. These results could occur under three possible conditions:

1.   The two *S. fimicola* strains have mutant alleles for the same gene (same locus on the same chromosome). Therefore, the same enzyme is affected in both mutants.

2.   The two *S. fimicola* strains have mutant alleles for different genes <u>on the same chromosome</u>. One strain is mutant for a gene on one side of the centromere; the other strain is mutant for a gene on the other side of the centromere. (The two genes are on opposite arms of the same chromosome, but at the same distance from the centromere.)

3.   The two *S. fimicola* strains are mutant at different genes <u>on different chromosomes</u>, but at the same distance from the centromere.

In conditions 2 and 3, different enzymes are affected by the mutation in the different strains.

In an attempt to determine which condition existed, the researcher crossed the two mutant strains.

C.   In the following table indicate in which of the 3 possible conditions the mutant x mutant cross would (or would not) produce asci with some black (wild type) spores. Briefly explain your answers.

Condition	Would the mut x mut cross produce black spores? (**yes** or **no**)	Explain how black spores would be formed, or why they would not be formed
1. 2 strains are mutant at same gene on same chromosome		
2. 2 strains are mutant at different genes on same chromosome		
3. 2 strains are mutant at different genes on different chromosomes		

D. The researcher has asked you to determine if her estimated map distances differ significantly from published map units. When reviewing literature on colorless spore *Sordaria fimicola* mutants, you find that the published distance between a colorless spore gene and the centromere is 22 map units (that is the frequency of crossover is 44% and the frequency of noncrossover is 56%).

Use the table below and the table of X^2 values on the last page of the exam to compare the data collected from the mutant x wild type cross to the data expected from the published map unit distance.

Categories	Observed	Expected	O-E	$(O-E)^2$	$\dfrac{(O-E)^2}{E}$
Non-X-over asci					
X-over asci					

Test X^2 = _____

Tabular X^2 value = _____ (_____ degrees of freedom, α = _____)

Conclusion based on results of test (state this as completely and precisely as you can):

19   **13.** Note: hatched bands on the gel are very faint; gray represents a "smear" of DNA; numbers above bands are their migration distances in mm.
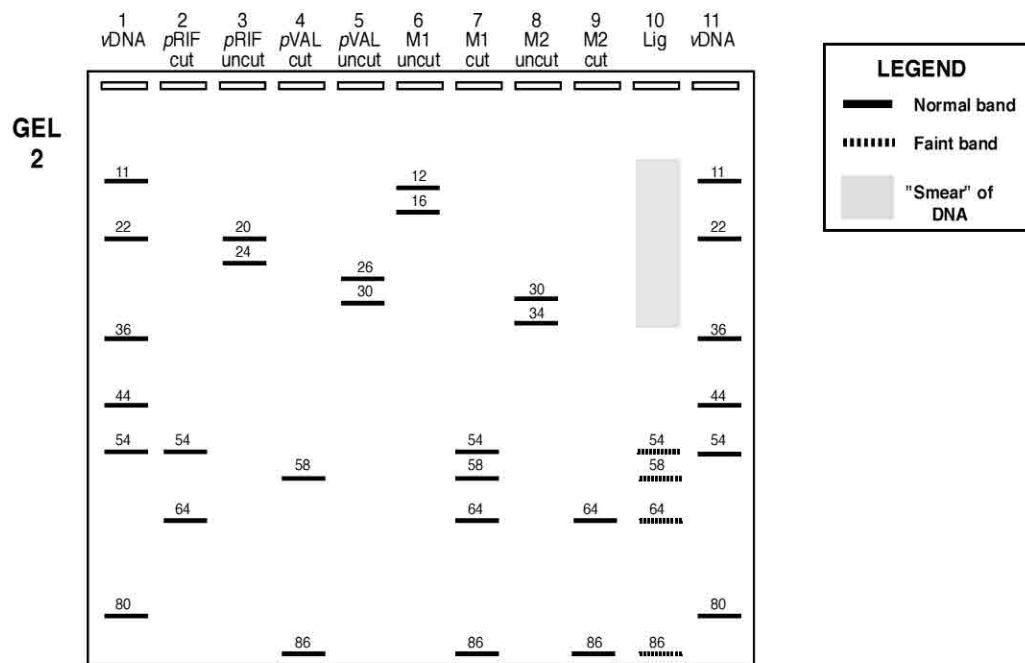
In this study, you use the procedures of Cohen, Chang, Boyer, Helling to create a recombinant plasmid from the plasmids *p*RIF (with a rifamycin resistance gene), and *p*VAL (with an valinomycin resistance gene). First, you cut each plasmid with two sticky-end producing restriction endonucleases, *Not*I and *Hind*III. The restricted fragments are combined with DNA ligase and ATP to create recombinant plasmids. Wild type *E. coli* are transformed with the ligation mixture and selection plating with valinomycin and rifamycin is used to identify the bacterial phenotypes. At each step in the procedure, you reserve a portion of the reaction mixture for later electrophoresis. The wells in the gel you run at the end of the study contain the following:

Well   Contents (abbreviation)

1, 11   Viral DNA (10,000 bp) cut with *Eco*RI yielding 6 size standard fragments (4100 bp, 2115 bp, 1485 bp, 1150 bp, 800 bp, and 350 bp) — (*v*DNA)

2   *p*RIF restricted with *Not*I and *Hind*III — (*p*RIF cut)

3   Unrestricted *p*RIF — (*p*RIF uncut)

4   *p*VAL restricted with *Not*I and *Hind*III — (*p*VAL cut)

5   Unrestricted *p*VAL — (*p*VAL uncut)

6   Miniprep 1, unrestricted — (M1 uncut)

7   Miniprep 1, restricted with *Not*I and *Hind*III — (M1 cut)

8   Miniprep 2, unrestricted — (M2 uncut)

9   Miniprep 2, restricted with *Not*I and *Hind*III — (M2 cut)

10   Ligation mixture (DNA ligase, ATP, and restricted *p*RIF and *p*VAL) — (Lig)

A.   List below all the numbers of the wells that received some DNA in the <u>supercoiled, circular</u> conformation.

B.   Using the appropriate band migration distance in mm shown above each band on the gel and the standard curve on **the last page of the exam**, estimate the plasmid fragment sizes, draw restriction maps showing all restriction sites and fragment sizes, and indicate the total size of both plasmids on the circles shown above.

*p*RIF
_____bp

*p*VAL
_____bp
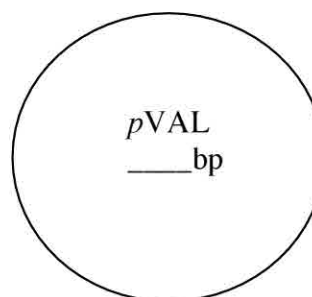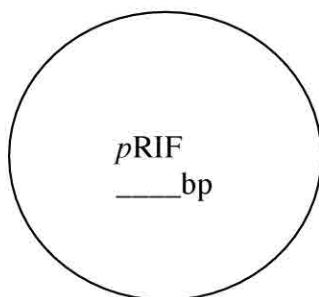
C.   How complete was the ligation? Be specific in your explanation.

D. The following table shows colony numbers on antibiotic-containing plates inoculated with non-transformed wild type *E. coli* or *E. coli* transformed with the ligation mixture.

Plate Contents	Wild type *E. coli*	Transformed wild type *E. coli*
No antibiotics	lawn	lawn
Rifamycin	0	542
Valinomycin	0	450
Rifamycin + Valinomycin	0	64

1. Estimate the number of colonies growing on the **rifamycin** antibiotic plate that possess the valinomycin resistance gene.

2. Each plate was inoculated with 100 μl of the transformation mixture. Approximately how many cells in the inoculum had acquired <u>only</u> a valinomycin resistance gene?

E. Two different colonies were scraped off the double antibiotic plate, each was placed in liquid growth media, and allowed to form a dense culture. Plasmid DNA was extracted from the cells in each culture and purified as miniprep DNA samples 1 and 2. Unrestricted DNA from miniprep samples 1 and 2 was placed into wells 6 and 8. DNA from miniprep samples 1 and 2 was restricted with *Not*I and *Hin*dIII *and* placed into wells 7 and 9.

Based on an examination of lanes 2, 4, 7 and 9 on which plasmid fragments are the two antibiotic resistance genes located. Be specific and refer to fragments by their sizes.

---

12   14. Below are 2 columns listing: 1) phyla, and 2) life cycle generations of plants you have studied. In all parts of this question you are asked to **identify** the specimen displayed. To do so, write the letters corresponding to the phylum and life cycle generation to which the specimen belongs (a minimum of 2 letters). If both gametophyte and sporophyte generations are known to be present in/on on the specimen, write both letters g and s.

Phylum
a. Anthophyta
b. Bryophyta
c. Coniferophyta
p. Pterophyta

Generation of life cycle
g. gametophyte
s. sporophyte

A. 1. Identify the specimen. _____

2. This specimen produced and carried which of the following items? (Circle <u>all</u> correct answers.)

megaspores   microspores   ovules   pollen grains   seeds

B. Identify the specimen. _____

C. Identify the specimen. _____

D. 1. Identify the specimen. _____

    2. This specimen is a: (circle correct answer.)

       fruit   megasporangium   ovule   seed

E. 1. Identify the specimen. _____

    2. This specimen produced and carried which of the following items? (Circle <u>all</u> correct answers.)

       megaspores   microspores   ovules   pollen grains   seeds

F. Assume the flower is real and the granules on the anthers are pollen.

    1. Identify the specimen. _____

    2. Give the letter of the structure that contained microspores. _____

    3. Give the letter of the structure that becomes a fruit or part of a fruit in all flower types. _____

6 _____ **15.** Use the diagram of a bean seedling at the right to answer the questions. For all the answers give numerals indicating the appropriate part(s) of the seedling.

A. Which part was the largest component of the bean seed? _____

B. Which part grows unevenly to form a "hook" as the seed germinates? _____

C. If the seedling had developed in total Darkness:

    1. Which part(s) would have been larger? _____

    2. Which part(s) would have been smaller? _____
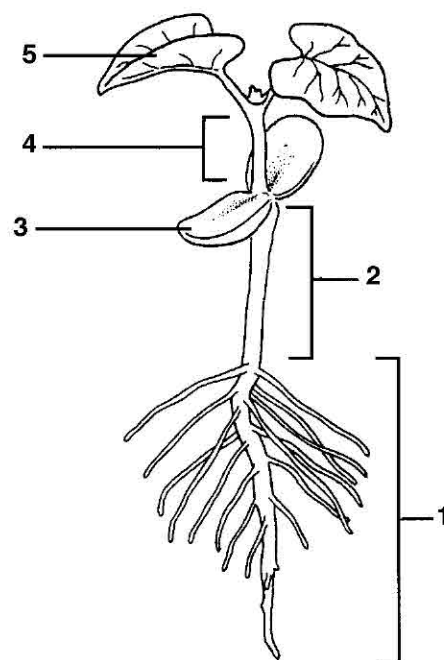
D. The size of which part decreases as the seedling continues to grow? _____

E. What is the ploidy of the structure labeled 3? (Circle your answer.)

       haploid   diploid   triploid

<u>8</u>   **16.**   A. Identify the indicated structures.

1._____

3._____

6._____

7._____
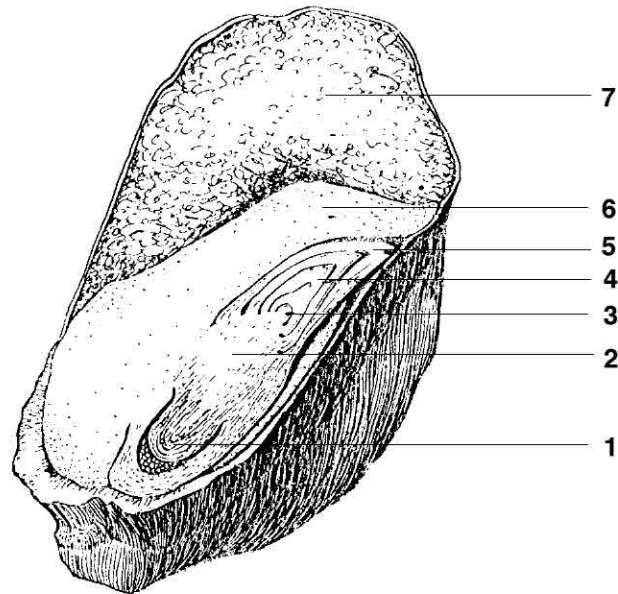
         B.   What is the function of the seedling part indicated by line 6?

         C.   What is the function of the seed structure indicated by line 7?

<u>4</u>   **17.**   Write the main dispersal structure for each phyla.

         Coniferophyta:_____

         Bryophyta:_____

         Anthophyta:_____

         Pterophyta:_____

<u>7</u>   **18.**   A bioassay for gibberellins is based upon the ability of gibberellins to stimulate the synthesis of the enzyme amylase in cereal grains such as barley. Barley half grains without embryos are incubated in test solutions and solutions of known gibberellin concentrations. The amylase produced hydrolyzes starch in the half grains to sugar, which is released into the incubation medium. The amount of sugar in the medium is proportional to the amount of amylase in the half grains.

$$\text{starch} \xrightarrow{\text{amylase}} \text{sugar}$$
$$\text{(in half grain)} \qquad\qquad \text{(in incubation medium)}$$
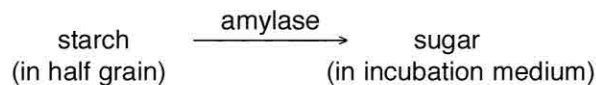
The figure below shows the dose-response curve from a bioassay using gibberellic acid (GA$_3$).
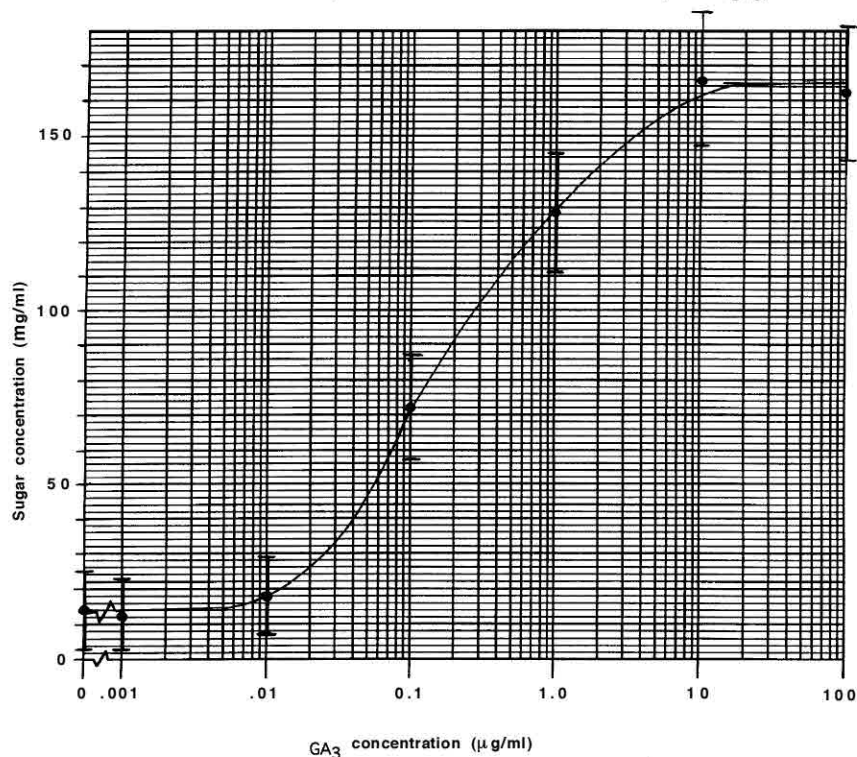
Figure 1. Dose-response curve showing variation in sugar concentration with different concentration of GA$_3$ in the incubation medium of barley half grains. Black dots are mean sugar concentrations from 10 replicates of each treatment. Vertical bars are ± 1 standard deviation from the mean.

Gibberellins were extracted from a liquid culture of the fungus *Gibberella fujikuroi*, and concentrated in a solution regarded as the undiluted extract (E). One-tenth, 1/100 and 1/1000 dilutions of E were prepared and aliquots of all four solutions were used in the barley half-grain bioassay. The results of 10 replicates of each solution are given below:

Solution	sugar conc.± 1 s.d. (mg/ml)
E undiluted	160±12
E diluted 1/10	155±10
E diluted 1/100	100±7
E diluted 1/1000	20±8

A. Which extract solution gave a sugar concentration that is best for estimating the gibberellin concentration of the extract?

B. Explain why your answer to A is best, and why the other E solutions are not.

C. What is the estimated concentration of GA$_3$-like substances in the <u>undiluted</u> extract? Show your calculations; underline your answer.

<u>12</u> **19.** A. From the lists of terms given below, complete the following table for the organisms. For each organism, select the letter(s) corresponding to the pond **region** (zone) where that organism would be found, the organismal **category** of which it is a member, and the **sampling method** used to collect it. **Note**: give the letters corresponding to <u>all</u> the terms that apply under a heading.

REGIONS
A. Limnetic
B. Littoral
C. Aphotic (Profundal)

CATEGORIES
A. Benthos
B. Nekton
C. Neuston
D. Periphyton
E. Phytoplankton
F. Zooplankton

SAMPLING METHODS
A. Dip net
B. Minnow trap
C. Periphyton slides
D. Plankton net

	ORGANISM	REGION	CATEGORY	SAMPLING
1)	Diatoms			
2)	Crayfish			
3)	Clam			
4)	Leech			

For question 12: Chi-square tabular values indexed according to alpha (0.05 to .001) and the degrees of freedom.

Degrees of Freedom	Probability of Obtaining a Value as Large or Larger		
	.05	.01	.001
1	3.841	6.635	10.827
2	5.991	9.210	13.815
3	7.815	11.345	16.268
4	9.488	13.277	18.465
5	11.070	15.086	20.517
6	12.592	16.812	22.457
7	14.067	18.475	24.322
8	15.507	20.090	26.125
9	16.919	21.666	27.877
10	18.307	23.209	29.588

Standard Curve for Question 13
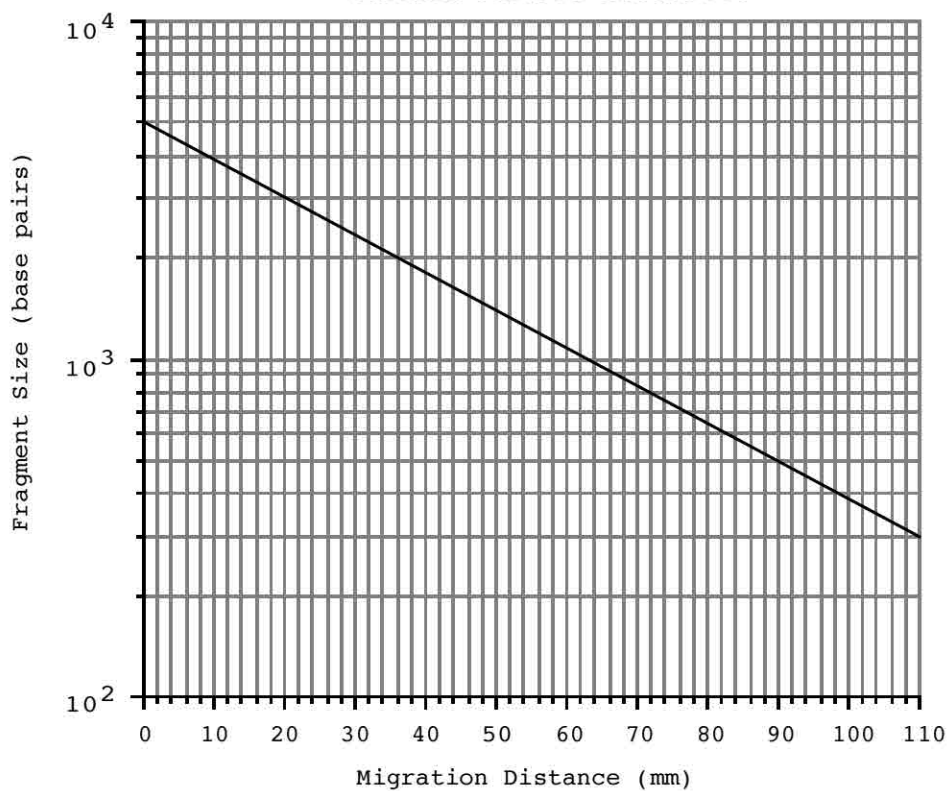
BioG 1108 Summer Lab Practical Answer Key

1. (common name and phylum: 0.5 points each; descriptive terms 0.25 points each)

Slide	Common Name	Phylum	Letters of Four Descriptive Terms
1	leech	Annelida	A F I L
2	rotifer	Rotifera	B FI J
3	nematode	Nematoda	B F I J
4	Clam	Mollusca	A F I K

2. (3 points) Choices: 1a, 2b, 3b, 4a, 5a
(1 point) Order: Coleoptera
(3 points) any morphological adaptation for aquatic living, such as a streamlined body shape

3. (0.5 points each answer)

Room 1108	Room 1112	structure	function
3	4	Clitellum	Secretes mucus during reproduction
5	6	Aortic arch	Pumps blood to ventral blood vessel
7	8	Seminal vessicle	Stores sperm prior to mating
8	1	Gizzard	Grinds food

Structure: circular muscles
Function: decreases the circumference and increases the length of a segment

4. (0.5 points each answer)

Dividing Cell is…	Diploid		Haploid	
	Double Chromatid Chromosomes	Homologous Pairs	Double Chromatid Chromosomes	Homologous Pairs
Mitosis Prophase	+	+	+	−
Mitosis Telophase	−	+	−	−
Meiosis Prophase I	+	+	NA	NA
Meiosis Prophase II	+	−	NA	NA
Meiosis Telophase II	−	−	NA	NA

5A. (1 point) 1108: male, 1112: female
5B. (0.5 points each)

	structure	function
1	Gill	Respiration
2	Hepapopancreas	Secretes digestive enzymes
3	Cheliped	Grasping and tearing food, defense
4	Green gland	Excretion/osmoregulation

6. (5 points) pipetting question

7A. (1 point) Possible answers include a) as in vertebrates, the squid's circulatory system is closed. b) The squid has a separate heart for pumping blood to the gills and body. This is similar to the mammalian condition in which the left ventricle pumps to the body and the right ventricle pumps to the lungs.

7B. (0.5 points each)

	structure	function
1	Funnel	Jet propulsion as water is forced out
2	Tentacle	Used to grasp prey
3	Rectum	Excretion
4	Nidamental Gland	Secretes covering for egg capsules

8A. (1 point) excurrent siphon

8b. (0.5 points each)

	structure	Function
1	Mantle	Secrete shell, enclose gills, form siphons
2	Gills	Respiration and filter feeding (feeding was necessary for full credit)
3	Foot	Movement, burrowing
4	Posterior adductor muscle	Muscle that closes shells

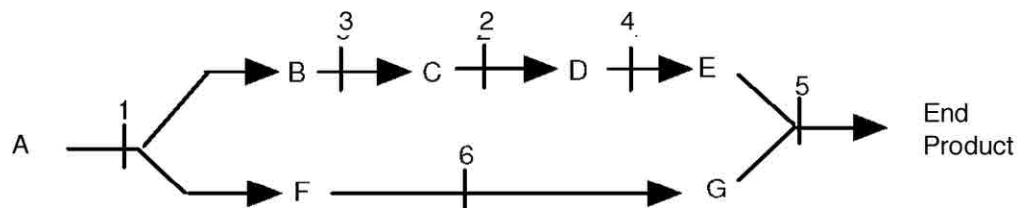9A. (2 points) a) Only strain I will be purple

9B. (3 points) Strain I secretes intermediates B and H and cannot feed Strain II. Strain II secretes E, H, and D and can feed Strain I. Therefore, only strain I can complete the pathway and will make the purple end product.

10A. (1 point) strain 1
10B. (1 point) strain 5
10C. (1 point) strains 2 and 6; 3 and 6; 4 and 6
10D. (5 points)

11A. (0.5 points each)

.	_D_	Acrosomal enzymes that digest jelly coat	8.	_I_	Egg cytoplasm
.	_G_	Acrosomal process	9.	_F_	Egg plasma membrane
.	_B_	Acrosome	10.	_N_	Fertilization membrane
.	_C_	Actin filaments	11.	_K_	Jelly coat
.	_A_	Bindin receptor sites	12.	_M	Perivitelline space
.	_J_	Contents of cortical granule	13.	_L_	Sperm nucleus
.	_E_	Cortical granule	14.	_H_	Vitelline layer

11B. (2 points) The bindin receptor proteins on the egg are specific for bindin proteins on the sperm cells from individuals of the same species. Sperm from incompatible species are unable to bind to the egg.

11C. (2 points)This is particularly important to urchins, where fertilization is external and both egg and sperm are released into the environment where they are more likely to be exposed to sperm and eggs from other species of aquatic organisms. Fertilization in the crayfish is accomplished when the male transfers a sperm package directly to the seminal receptacle of the female. Although fertilization is external, there is more control in guiding sperm to the appropriate species of egg (crayfish gametes do have bindin and bindin receptors).

12A. (2 points) 400/1000 = 0.4 12B. (

12B. (2 points) (400 • 4)/(1000 • 8) • 100 = 20 map units

12C. (2 points each)

   1. No. Each strain is mutant for the same gene, so all spores will be albino.

   2. Yes. During meiosis, some crossovers between each gene locus and the centromere will result in some spores with wild type alleles for both genes on the same chromatid.

   3. Yes. Independent assortment during meiosis will result in some spores with chromosomes with both wild type alleles. 12D. (6 points)

| Categories	O	E	O – E	$(O – E)2$	$(O – E)2 / E$
Non-X-over asci	600	560	40	1600	2.86
X-over asci	400	440	-40	1600	3.64

$$X^2 = 6.5$$

Tabular $X^2 = 3.841$ (1 d. f.; $\alpha = 0.05$)

Reject H_O; there are significant differences between the observed numbers of noncrossover and crossover asci and the numbers expected if the mutant gene-centromere map distance is 22 map units. Therefore, the calculated map unit distances are different from published map unit distances.

13A. (2 points) Wells 3, 5, 6, 8, 10

13B. (5 points)
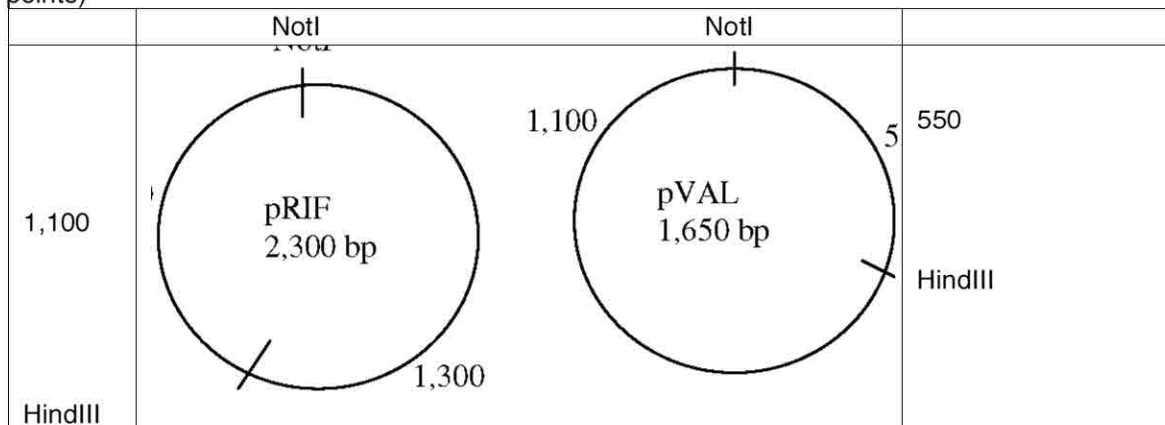
13C. (2 points) Ligation was incomplete because some trace of the four bands representing the restriction fragments seen in lanes 2 and 4 are still visible in lane 10.

13D1. (1 point) 64 colonies

13D2. (1 point) 450 – 64 = 386 cells

13E. (4 points) The valinomycin resistance gene is located on the 550 bp *p*VAL fragment; the rifamycin resistance gene is located on the 1,000 bp *p*RIF fragment. These two bands are present in both miniprep lanes.

14A. (2 points) 1.c g s; 2. megaspores, ovules, seeds
14B. (1.5 points) p g s
14C. (1.5 points) b g
14D. (2 points) 1. a s; 2. fruit
14E. (2 points) 1. c g s; 2. microspores, pollen grains
14F. (3 points) 1. a g s; 2. room 1108: A, room 1112: B; 3. room 1108: D, room 1112: A

15A. (1 point) 3
15B. (1 point) 2
15C. (2 points) 1. 2 and 4 would have been larger; 2. 5 and 3 would have been smaller
15D. (1 point) 3
15E. (1 point) diploid

16A.   (1 point each) 1. radicle; 3. plumule; 6. cotyledon; 7. endosperm
16B.   (2 points) It absorbs nutrients from 7 (endosperm) and transfers them to the growing parts of the embryo.
16C.   (2 points) provides nutrition to the developing embryo

17.   (1 point each). Coniferophyta: seed
      Bryophyta: spore
      Anthophyta: seed
      Pterophyta: spore

18A. (2 points) E diluted 1/100.
18B. (3 points) The magnitude of the response produced by E diluted 1/100 is between the upper and lower limits of the response range in the bioassay. The effect of E diluted 1/1000 is no greater than the effect of the control treatment. E diluted 1/1000 is near or below the threshold concentration. The magnitude of the response to E undiluted and E diluted 1/10 is at the upper limit of the response range which results from a concentration (dose) that saturates the bioassay system. The concentration of GAs in E and E 1/10 could be any value near or above the minimal saturation concentration.
18C. (2 points) The response to E diluted 1/100 is 100 + 7 mg/ml sugar which corresponds to 0.3 ug/ml GA3 like substances. The concentration in E undiluted is 100 times greater (30 μg/ml).

19. (12 points total; 1 point in each cell, 0.25 was deducted for a wrong answer)

	ORGANISM	REGION	CATEGORY	SAMPLING
1)	Diatoms	A B	D E	C
2)	Crayfish	B C	A	A B
3)	Clam	B C	A	A
4)	Leech	B	A	A